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Studies on the endogenous L-selectin ligands: systematic and highly efficient total synthetic routes to lactamized-sialyl 6-*O*-sulfo Lewis X and other novel gangliosides containing lactamized neuraminic acid☆

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Abstract

Systematic syntheses of lactamized neuraminic acid-containing gangliosides GM4, sulfated sialylparagloboside, and sulfated/nonsulfated sialyl Lewis X are described. The highly efficient, one-step lactamization of neuraminic acid was accomplished by treatment of the *N*-deacetylated sialic acid (neuraminic acid)-containing gangliosides with HBTU and HOBt in DMF at 65 °C. Both the lactamized neuraminic acid residue and the sulfate group at O-6 of the GlcNAc residue were found to be involved in the antigenic determinant defined by G159 monoclonal antibody, while the fucose residue may not be critical for the recognition by G159 mAb.

Keywords: Selectin; Sialic acid; Sialyl Lewis X; Ganglioside; Oligosaccharide; Glycosylation

1. Introduction

Selectins (L-, E- and P-selectin)[2] are a family of cell-adhesion molecules (C-type lectin) having an N-terminal carbohydrate recognition domain that play important roles in the homing of lymphocytes, recruitment of leukocytes to sites of inflammation, thrombosis, cancer metastasis, and so on. The sialyl Lewis X (sLe^x)[3] has been recognized as a common, minimal effective structure for binding to the three selectins.[4] Recently, it has been demonstrated[5] with chemically synthesized gangliosides[6] that the novel sLe^x variant (sialyl 6-*O*-sulfo Le^x, [**A**] in Fig. 1) sulfated at O-6 of the *N*-acetylglucosamine (GlcNAc) residue in sLe^x is an endogenous L-selectin ligand on the human high endothelial venule (HEV) by using two kinds of monoclonal antibodies[7] (G152 and G72 mAbs). Very recently, it has been suggested that the interactions of trophoblast L-selectin with the uterus may be specifically mediated by sialyl 6-*O*-sulfo Lewis X and that the adhesion mechanism may be critical to establishing human pregnancy.[8] Also, the *N*-deacetylated form[5,9] of [**A**] ([**B**] in Fig. 1) was found to be a superior ligand for L-selectin, which may be inactivated (down regulation) by the enzymatic cyclization to give the lactamized form[10] ([**C**] in Fig. 1), specifically detected with G159 monoclonal antibody. These novel series of sulfated sLe^x variants were originally discovered as synthetic byproducts,[5b,5c] but later it was shown that these novel structures may be widely expressed on a variety of leukocytes, giving rise to a new regulation mechanism in the homing of lymphocytes based on the heterogeneity of L-selectin ligands.[10,11] The chemical structure of [**C**] has been estimated by combination of analytical (MS,[5b,12] NMR[13]) and synthetic[14] approaches, but the full

☆ Synthetic studies on sialoglycoconjugates, Part 135. For Part 134, see Ref. [1].

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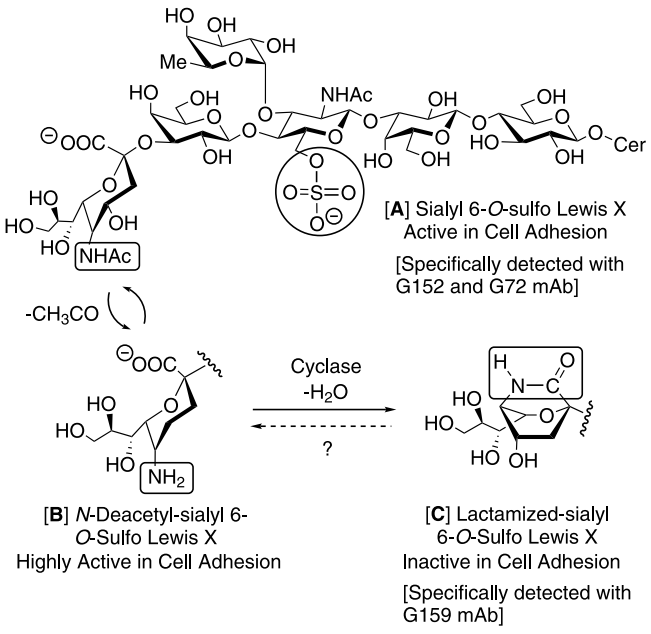


Fig. 1. Hypothetical metabolic pathway of sialyl 6-*O*-sulfo Lewis X as an endogenous ligand for L-selectin.

characterization by a completely stereocontrolled total synthesis of the pure ganglioside composed of lactamized-sialyl 6-*O*-sulfo Le^x hexasaccharide has not been successful so far. In addition, the details of the determinant defined by G159 mAb have also remained obscure.

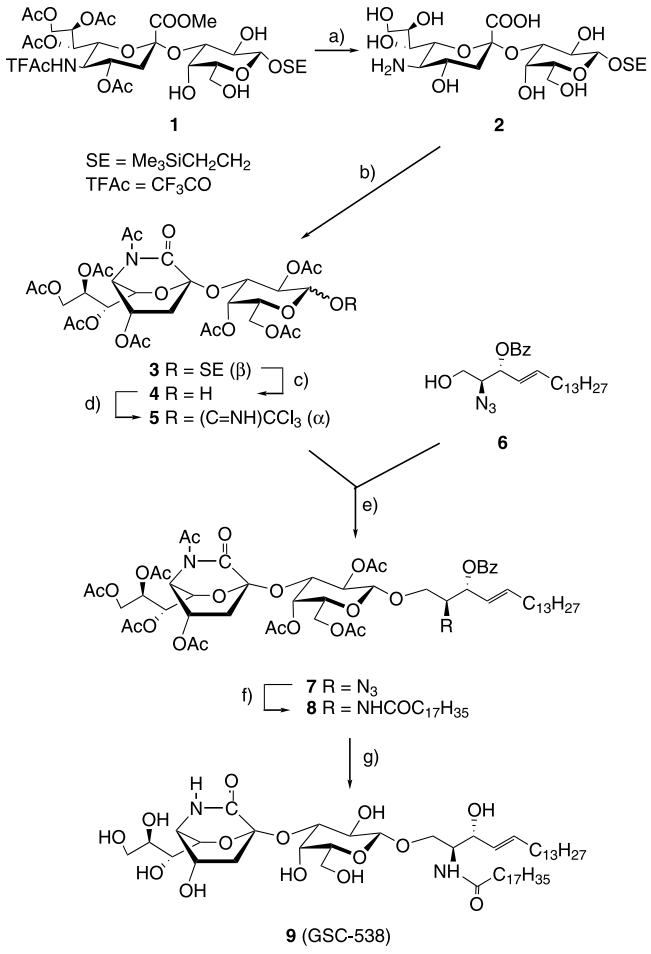
In our previous communications,[14] it has been suggested that the lactamization may be achieved by treatment of the *N*-deacetylated form with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (WSCHCl) in dimethyl sulfoxide (DMSO) at 60 °C. However, the yields of the desired sulfated sLe^x derivatives were very low and accompanied by many unknown byproducts.

We here report the systematic and highly efficient total synthetic routes to novel gangliosides GM4 (**9**), sulfated sialylparagloboside (SPG) (**23**), and sulfated/nonsulfated sialyl Lewis X (**37**, **38**) containing lactamized neuraminic acid. The antigenic reactivities of the synthetic gangliosides with the G159 monoclonal antibody are also described.

2. Results and discussion

2.1. Synthesis

We first examined the efficient lactamization of neuraminic acid by using 2-(trimethylsilyl)ethyl 5-amino-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid-(2→3)-β-D-galactopyranoside (**2**), which can be readily prepared from **1**[15] (Scheme 1). When **2** was reacted with a mixture of 1,3-dicyclohexylcarbodiimide



Scheme 1. Total synthetic route to lactamized GM4 ganglioside **9**. (a) NaOMe, MeOH, then H_2O, 45 °C, 99%; (b) 1. DCC, HOBt, DMF, 65 °C; 2. Ac_2O, Pyr., 53%; (c) TFA, CH_2Cl_2, rt, 95%; (d) CCl_3CN, DBU, CH_2Cl_2, 0 °C, 94%; (e) TMSOTf, AW-300, CH_2Cl_2, 0 °C, 49%; (f) 1. H_2, Pyr.–H_2O, 0 °C; 2. $C_{17}H_{35}CO_2H$, WSC, CH_2Cl_2, rt, two steps, 38%; (g) NaOMe, MeOH–dioxane, rt, quant.

(DCC) and *N*-hydroxybenzotriazole (HOBt) in *N*,*N*-dimethylformamide (DMF) at 65 °C, followed by complete acetylation, the desired lactam derivative **3** was obtained in a satisfactory yield (53%), while the single use of DCC or WSC in DMF gave **3** in less than 10% yield. The 2-(trimethylsilyl)ethyl (SE) group in **3** could be cleaved by treatment with trifluoroacetic acid (TFA) in high yield (95%) as usual, and the resulting hemiacetal **4** was successfully converted to the trichloroacetimidate derivative **5** in 94% yield, suggesting that the N-acetylated lactam ring in **3** may be stable enough against both acid and base under anhydrous conditions.

Coupling of **5** with the azidosphingosine derivative **6**[16] gave **7** in 49% yield, and the successive reduction of the azido group and N-acylation were carried out by the established method[17] to afford **8** in 38% yield. In this course, unfortunately, the lactam ring was found to be labile against the reductive N-acylation process (**7**→**8**)

giving unknown by-products. This result suggested that the lactamization reaction should be conducted in the final stage after introduction of the ceramide moiety. Finally, removal of all protective groups in **8** under basic conditions furnished the desired lactamized GM4 ganglioside **9** in an almost quantitative yield.

Based on these results, we next examined the systematic and highly efficient synthetic routes to lactamized-sialyl 6-*O*-sulfo paragloboside (**23**), lactamized-sialyl Lewis X (**37**) and lactamized-sialyl 6-*O*-sulfo Lewis X (**38**) gangliosides through the corresponding, *N*-deacetyl-sialyl SPG (**22**)/Lewis X (**35**, **36**) gangliosides (Fig. 2). The first key step in this strategy is the efficient construction of the suitably protected SPG pentasaccharide intermediate, in which the amino group of sialic acid and O-6 of the GlcNAc residue are protected by the trifluoroacetyl (TFAc) and 4-methoxyphenyl (MP) groups, respectively. The TFAc protected sialic acid is equivalent to the N-deacetylated and lactamized sialic acids, and the MP group can be chemoselectively cleaved by ceric ammonium nitrate (CAN). The *N*-deacetyl-sialyl SPG/Lewis X can be synthesized directly from the corresponding SPG intermediate or by incorporation of fucose at O-3 of the GlcNAc residue in the SPG acceptor (**24**). The most important problem is how to efficiently construct the lactamized neuraminic acid from the *N*-deacylated form in the presence of a sulfate group at O-6 of the GlcNAc residue.

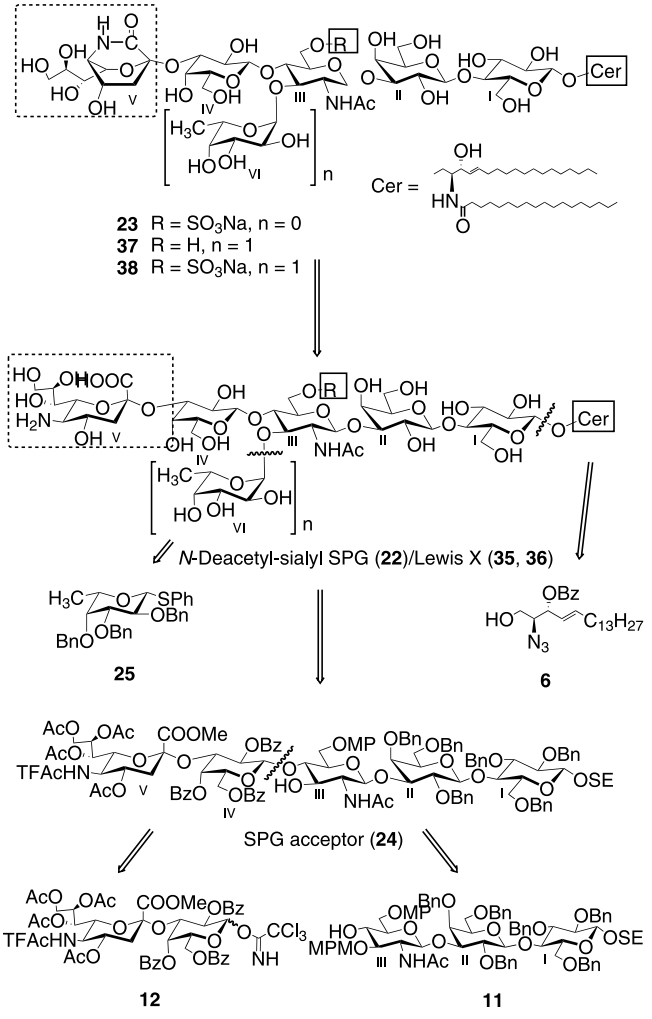
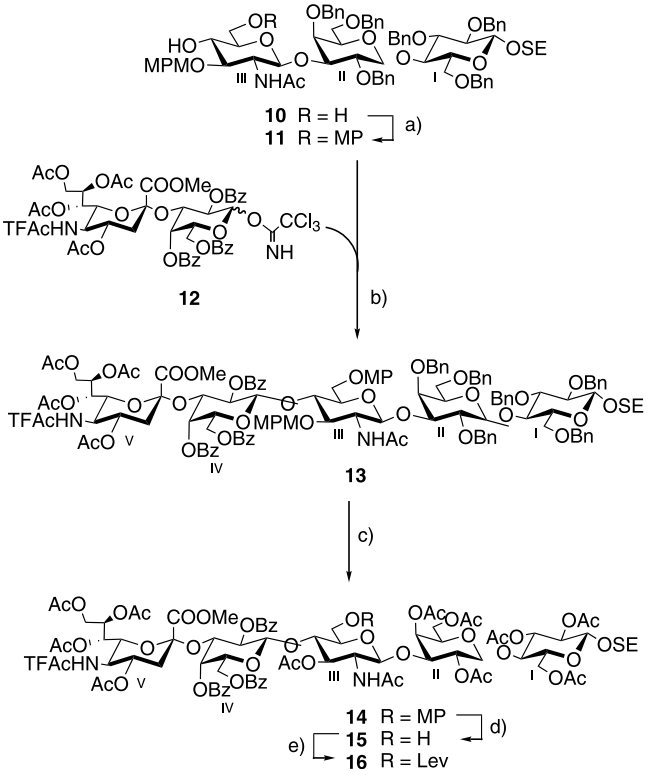


Fig. 2. Target structure and retrosynthetic analysis of lactamized-sialyl 6-*O*-sulfo paragloboside (**23**), lactamized-sialyl Lewis X (**37**), and lactamized-sialyl 6-*O*-sulfo Lewis X (**38**).

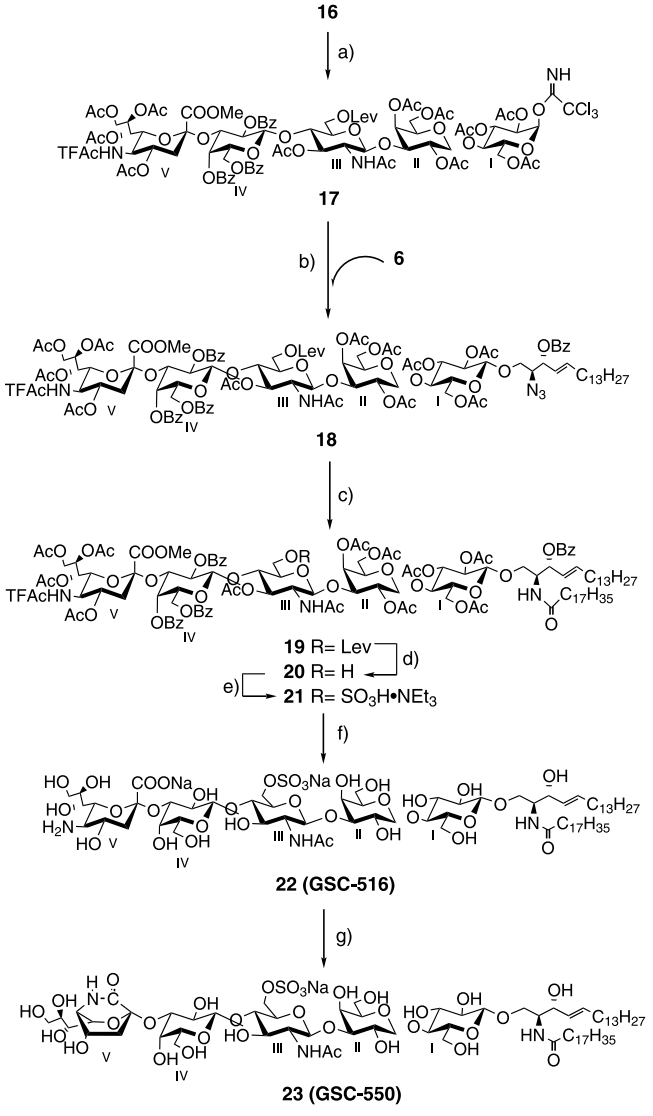
The regioselective 4-*O*-methoxyphenylation of O-6 of **10**[6] was carried out by treatment with *p*-methoxyphenol (MPOH), PPh_3 and diethylazodicarboxylate (DEAD) in THF,[18] and the resulting **11** was coupled with the suitably protected α-*N*-trifluoroacetylneuraminyl-(2→3)-galactose donor **12**,[15] promoted by trimethylsilyl trifluoromethanesulfonate (TMSOTf), to give the desired SPG pentasaccharide **13** in high yield (Scheme 2).

Hydrogenolytic removal of the benzyl (Bn) and 4-methoxybenzyl (MPM) groups in **13** and the following acetylation gave **14**. Since the ceramide moiety has been found to be labile against CAN that cleaves the MP group chemoselectively, the MP group in **14** was replaced[19] by the levulinoyl (Lev) group to afford **16**. The SE group in **16** was then selectively cleaved[20] by treatment with TFA, and the resulting hemiacetal was



Scheme 2. Synthesis of the suitably protected SPG intermediate. (a) MPOH, PPh_3, DEAD, THF, 80 °C, 76%; (b) TMSOTf, CH_2Cl_2, 4 Å MS, 0 °C, 81%; (c) 1. H_2, $Pd(OH)_2$; 2. Ac_2O, Pyr., two steps, 90%; (d) CAN, CH_3CN–H_2O, 0 °C, 99%; (e) Lev_2O, DMAP/Pyr., 60 °C, 73%.

converted[21] to the trichloroacetimidate derivative **17** in 95% yield. Coupling of **17** with **6**, and the subsequent reduction of the azido group in **18** and *N*-acylation were carried out as described for **8** (Scheme 3). Selective cleavage of the Lev group in **19** and the subsequent 6-*O*-sulfation of **20** with a sulfur trioxidepyridine (SO_3Pyr.) complex in DMF, followed by an addition of Et_3N to stabilize the sulfate group during the column chromatography, gave **21** in good yield. Removal of all protective groups in **21** under alkaline conditions afforded *N*-deacetyl-sialyl 6-*O*-sulfo paragloboside **22** (GSC-516), quantitatively, which upon treatment[22] with HBTU and HOBt in DMF at 65 °C to afford lactamized-sialyl 6-*O*-sulfo paragloboside **23** (GSC-550) in 96% yield. This result suggested that the one-step lactamization after introduction of the ceramide moiety and *N*-deacetylation of sialic acid seems to be most efficient as shown in Fig. 2.

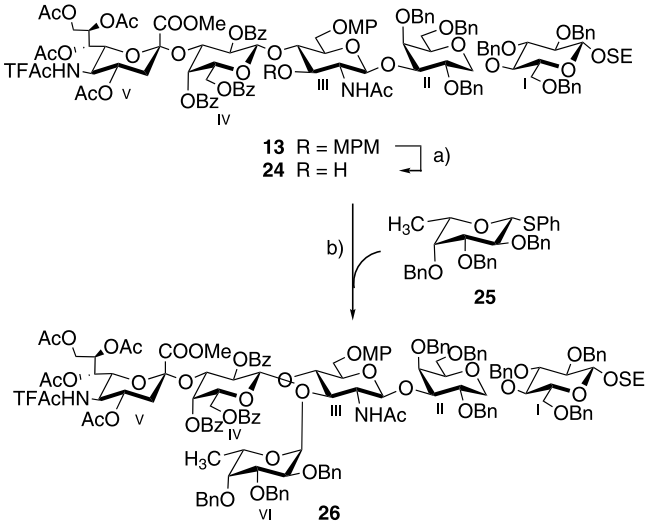


Scheme 3. Total synthetic route to lactamized-sialyl 6-*O*-sulfo paragloboside **23**. (a) 1. TFA–CH_2Cl_2, 0 °C, quant., 2. CCl_3CN, DBU–CH_2Cl_2, 0 °C, 95%; (b) TMSOTf–CH_2Cl_2, AW300, 0 °C, 21%; (c) 1. H_2S, Pyr., 0 °C; 2. WSC, stearic acid, rt, two steps, 66%; (d) NH_2NH_2AcOH–EtOH, 73%; (e) PyrSO_3 complex–DMF, then Et_3N, rt, 70%, (f) NaOMe–MeOH, 45 °C, quant.; (g) HBTU, HOBt, DMF, 65 °C, 96%.

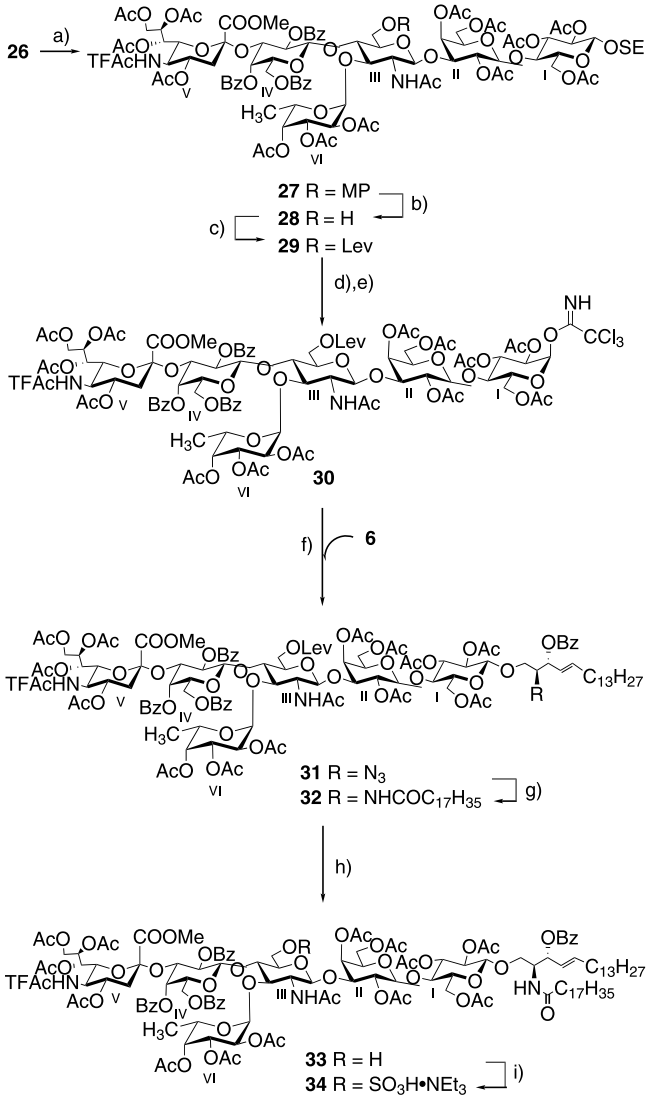
For the systematic syntheses of lactamized-sialyl Lewis X (**37**) and 6-*O*-sulfo Lewis X (**38**) gangliosides, the MPM group at O-3 of **13** was selectively removed by treatment with a mixture of TMSCl, $SnCl_2$ and anisole[23] in an almost quantitative yield to give the SPG acceptor **24**, which was then coupled with the fucose donor **25**,[6] affording the key hexasaccharide intermediate **26** in an excellent yield (Scheme 4). The ^{1}H NMR spectrum of this compound showed signals at δ 5.00 (d, 1H, $J_{1,2}$ = 3.2 Hz, H-1^{VI}), which are characteristic of the α-fucopyranosyl unit. Hydrogenolytic removal of the benzyl groups in **26** and the subsequent acetylation gave **27**.

The hydroxyl group at C-6 of the GlcNAc residue in compound **27** was re-protected by the levulinoyl (Lev) group as described for **16** to give **29**, which was then converted[20,21] to the trichloroacetimidate derivative **30** (Scheme 5). Coupling of **30** and the azidosphingosine derivative **6**,[16] and the subsequent conversion of the azido group in **31** to the stearoylamino group were carried out as described for **8** and **19** to afford **32**.

The selective cleavage of the Lev group at O-6 of the GlcNAc residue in **32**, and the subsequent 6-*O*-sulfation as described for **21** afforded **34** in 81% yield (Scheme 4). Removal of all protective groups of **32** and **34** under alkaline conditions gave the sulfated/nonsulfated *N*-deacetyl-sialyl Le^x gangliosides (**35**, **36**) almost quantitatively, which were finally lactamized in one step with HBTU and HOBt in DMF at 65 °C to afford lactamized-sialyl Lewis X (**37**, GSC-517) and lactamized-sialyl



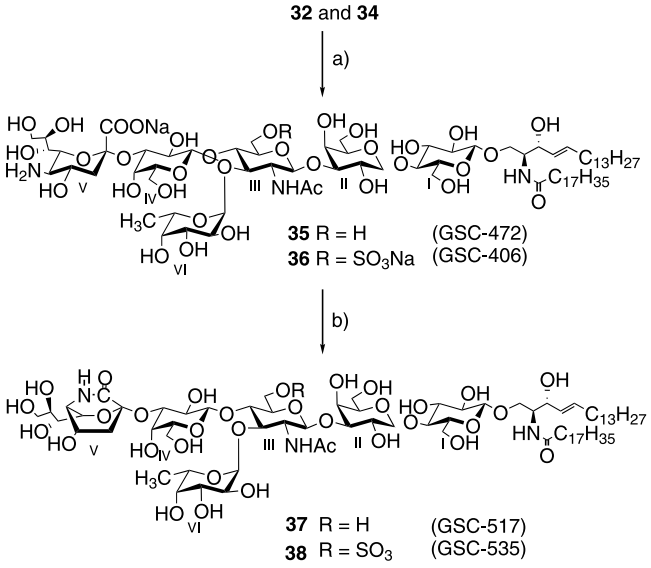
Scheme 4. Fucosylation of SPG acceptor **24**. (a) TMSCl, $SnCl_2$, anisole, CH_2Cl_2, 0 °C, 97%; (b) NIS, TfOH, benzene, 7 °C, 87%.



Scheme 5. Synthesis of the protected sialyl 6-*O*-sulfo Lewis X ganglioside. (a) H_2, $Pd(OH)_2$, AcOH–EtOH, then Ac_2O, Pyr., two steps, quant; (b) CAN, CH_3CN–H_2O, 0 °C, 85%; (c) Lev_2O, DMAP, Pyr., 60 °C, 82%; (d) TFA–CH_2Cl_2, 0 °C, quant.; (e) CCl_3CN, DBU, CH_2Cl_2, 0 °C, 87%; (f) TMSOTf, CH_2Cl_2, AW300, 0 °C, 69%; (g) H_2S, Pyr.–H_2O, 0 °C, then stearic acid, WSC, rt, two steps, 46%; (h) NH_2NH_2AcOH, EtOH, 70%; (i) SO_3Pyr. Complex–DMF, then Et_3N, rt, 81%.

6-*O*-sulfo Lewis X (**38**, GSC-535) gangliosides in 95% and 94% yields, respectively (Scheme 6).

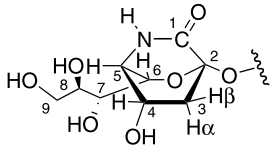
Table 1 shows the comparison of the selected ^1H NMR data for H-3α and H-3β of the totally synthesized, pure lactamized-sialyl (**23**, **37**, **38**) and *N*-deacetyl-sialyl (**22**, **35**, **36**) SPG/Lewis X gangliosides. In **22**, **35** and **36**, H-3α and H-3β have the typical axial and equatorial configuration, respectively, based on the $^2C_5$ chair conformation, showing the diaxial ($J_{3\alpha,4} = 12.1$ Hz) and axial–equatorial ($J_{3\beta,4} = 3.2$, 4.3 Hz) coupling constants, respectively. In contrast, in the lactamized forms (**23**, **37**, **38**), the values of vicinal couplings ($J_{3,4}$)



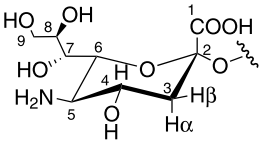
Scheme 6. Synthesis of lactamized-sialyl Lewis X (**37**) and lactamized-sialyl 6-*O*-sulfo Lewis X (**38**) gangliosides. (a) NaOMe, MeOH, 45 °C, then H_2O, quant.; (b) HBTU, HOBt, DMF, 65 °C, 95% (R = H), 94% (R = SO_3Na).

changed dramatically ($J_{3\alpha,4} = 4.1$–5.0 Hz, $J_{3\beta,4} = 10.3$–10.8 Hz) to indicate clearly that typical boat ($^{5,2}B$) type structures were formed by lactamization. These ^1H

Table 1
Comparison of the selected ^1H MNR data [a] of the neuraminic acid part (H-3α and H-3β) in the sulfated/nonsulfated lactamized-sialyl (**23**, **37**, **38**) and *N*-deacetyl-sialyl SPG (**22**)/Lewis X (**35**, **36**) gangliosides.



$^{5,2}B$ (**23**, **37**, **38**)



$^2C_5$ (**22**, **35**, **36**)

Compound no.	H-3α	H-3β
	δ (multiplicity, *J* (Hz))	δ (multiplicity, *J* (Hz))
23	2.01 (dd, $J_{gem} = 14.1$, $J_{3\alpha,4} = 5.0$)	2.29 (d, $J_{gem} = 14.1$, $J_{3\beta,4} = 10.5$)
37	2.01 (dd, $J_{gem} = 14.1$, $J_{3\alpha,4} = 4.1$)	2.29 (d, $J_{gem} = 14.1$, $J_{3\beta,4} = 10.8$)
38	2.00 (dd, $J_{gem} = 13.7$, $J_{3\alpha,4} = 4.6$)	2.29 (d, $J_{gem} = 13.7$, $J_{3\beta,4} = 10.3$)
22	1.62 (t, $J_{gem} = J_{3\alpha,4} = 12.1$)	2.74 (dd, $J_{gem} = 12.1$, $J_{3\beta,4} = 4.3$)
35	1.72 (t, $J_{gem} = J_{3\alpha,4} = 12.1$)	2.85 (dd, $J_{gem} = 12.1$, $J_{3\beta,4} = 3.2$)
36	1.79 (t, $J_{gem} = J_{3\alpha,4} = 12.1$)	2.81 (dd, $J_{gem} = 12.1$, $J_{3\beta,4} = 4.3$)

[a] Measured at 500 MHz in CD_3OD.

NMR data are well consistent with those reported previously.[13]

The negative-ion mass spectra of **37** and **38** gave the molecular ions at *m/z* 1630 $[M-H]^-$ and 1710 $[M-Na]^-$, respectively, and the fragment ions at *m/z* 1399 $[M-H\text{-Neu}]^-$, 1237 $[1399\text{-Gal}]^-$, 888 [lactosyl ceramide]$^-$, 726 [glucosyl ceramide]$^-$, 564 [ceramide]$^-$ for **37**, and at *m/z* 1479 $[M-Na\text{-Neu}]^-$, 1317 $[1479\text{-Gal}]^-$, 888, 726, 564 for **38**, respectively (Fig. 3). The ions at *m/z* 1399 and 1479 (-231 Da) correspond to the fragments obtained by glycosidic cleavage of the terminal lactamized neuraminic acid.

2.2. Antibody studies

During the course of generating monoclonal antibodies against synthetic sialyl 6-*O*-sulfo Lewis X ganglioside[6,7] ([**A**] in Fig. 1), we obtained an antibody G159 having reactivity against an unknown byproduct produced by treatment of *N*-deacetyl-sialyl 6-*O*-sulfo Lewis X ganglioside[5,9] ([**B**] in Fig. 1) with water-soluble carbodiimide (WSC) at 60 °C.[14] Therefore, the G159-defined determinant has been estimated to be a modified sialyl 6-*O*-sulfo Lewis X carrying a cyclic (lactamized) sialic acid.[10–12]

As shown in Fig. 4, the totally synthesized lactamized-sialyl 6-*O*-sulfo Lewis X ganglioside (**38**) was strongly stained with G159 antibody in TLC-immunostaining, while the nonsulfated analog **37** was not (data not shown). Interestingly, the lactamized-sialyl 6-*O*-sulfo paragloboside (**23**) was also clearly stained by G159 mAb, suggesting that the fucose moiety may not be critical for the recognition by G159 mAb. These results suggest that both the lactamized sialic acid residue and the sulfate group at O-6 of GlcNAc would be involved in the G159-defined determinant. The details of the recognition mapping defined by G159 mAb will be reported elsewhere.

3. Conclusions

In summary, we have succeeded for the first time in the highly efficient and completely stereocontrolled total syntheses of lactamized-sialyl 6-*O*-sulfo Lewis X (hexasaccharide) and other novel gangliosides containing lactamized neuraminic acid. Utilizing the synthetic ganglioside probes, we demonstrated that lactamized-sialyl 6-*O*-sulfo Lewis X is one of the major antigenic determinants defined by G159 mAb. We are now trying to characterize the enzymes involved in the metabolic pathway of sialyl 6-*O*-sulfo Lewis X as a L-selectin ligand. The distribution and biological functions of these carbohydrate antigens and related enzymes are now under investigation

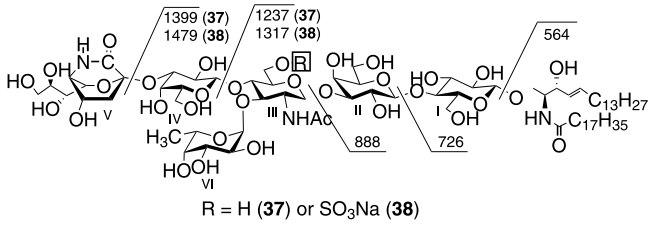


Fig. 3. Major fragmentation patterns in the FAB negative-ion mass spectra of **37** (R = H) and **38** (R = SO_3Na).

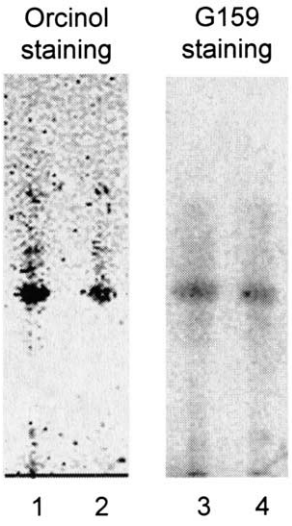


Fig. 4. TLC-immunostaining patterns of lactamized-sialyl 6-*O*-sulfo Lewis X ganglioside **38**. Left panel, orcinol–H_2SO_4 staining; right panel, immunostaining patterns of the same TLC plate with the G159 antibody. The amount of **38** applied was 2 μg in lanes 1 and 3, and 1 μg in lanes 2 and 4.

4. Experimental

4.1. General methods

TLC was conducted on E. Merck Silica Gel 60 F-254 aluminum plates. Compounds were visualized either by exposure to UV light or by spraying with a solution of 10% H_2SO_4 in EtOH. Column chromatography on silica gel (Fuji Silysia Co., 300 mesh) was performed with the solvent systems (v/v) specified. Specific rotations were determined with a Horiba SEPA-300 high-sensitive polarimeter at 25 °C. ^1H NMR and ^{13}C NMR spectra were recorded at 300 K with a Varian Unity Inova 500 (500 MHz) or Varian Unity Inova 400 (100.6 MHz) spectrometer, respectively. The values of δ (ppm) are given relative to Me_4Si as the internal standard. FABMS spectra were recorded on a JEOL JMS-SX 120A mass spectrometer/JMA-DA 7000 data system. CH_2Cl_2, MeOH, EtOH, benzene and DMF were kept dry over 4 Å MS, while pyridine and MeCN were kept dry over 3 Å MS.

4.2. 2-(Trimethylsilyl)ethyl 5-amino-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid-(2→3)-β-D-galactopyranoside (2)

To a solution of **1** (358 mg, 0.44 mmol) in MeOH (4 mL) was added 0.5 mL of 28% NaOMe in MeOH, and the mixture was stirred for 72 h at 45 °C. Water (0.5 mL) was added and the mixture was stirred for 24 h at rt. The mixture was neutralized with Amberlite IR-120 (H) resin and filtered. The resin was washed with MeOH, and the combined filtrate and washings were concentrated. Column chromatography (MeOH) of the residue on Sephadex LH-20 gave the target molecule (232 mg, 99%) as an amorphous mass; $[\alpha]_D$ −20.2° (*c* 1.7, MeOH); ^{1}H NMR (CD_3OD): δ 4.27 (d, 1 H, $J_{1,2}=8.4$ Hz, H-1^I), 2.85 (dd, 1 H, $J_{3eq,4}=4.3$ Hz, $J_{gem}=12.1$ Hz, H-3^{II}*eq*), 1.74 (t, 1 H, $J_{gem}=J_{3ax,4}=12.1$ Hz, H-3^{II}*ax*), 1.03 (m, 2 H, $Me_3SiCH_2CH_2$). Anal. Calcd for $C_{20}H_{39}NO_{13}Si$ (529.22): C, 45.36; H, 7.42; N, 2.64. Found: C, 45.20; H, 7.31; N, 2.51.

4.3. 2-(Trimethylsilyl)ethyl 5-acetylamino-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl 1,5-lactam-(2→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranoside (3)

To a solution of **2** (232 mg, 0.44 mmol) in DMF (4 mL) was added DCC (153 mg, 0.041 mmol) and HOBt (118 mg, 0.87 mmol), and the mixture was stirred for 24 h at 65 °C, and then concentrated. Column chromatography (MeOH) of the residue on Sephadex LH-20 gave lactamized sialyl galactose. The residue was treated with acetic anhydride (4 mL) and pyridine (6 mL) for 12 h at rt, then cooled to 0 °C. MeOH (3 mL) was added, the mixture was concentrated, and the residue was extracted with $CHCl_3$ and successively washed with cold 2 M HCl and water, dried (Na_2SO_4) and concentrated. Column chromatography (150:1 $CHCl_3$–MeOH) of the residue on silica gel gave **3** (196.8 mg, 53%, two steps) as an amorphous mass; $[\alpha]_D$ +31.4° (*c* 0.43, $CHCl_3$) ^{1}H NMR ($CDCl_3$): δ 5.75 (dd, $J_{7,8}=4.1$, $J_{6,7}=9.8$ Hz, H-7^{II}), 5.36 (m, 1 H, H-8^{II}), 5.18 (d, 1H, H-4^I), 5.11 (dd, $J_{1,2}=8.0$, $J_{2,3}=10.0$ Hz, H-2^I), 4.81 (m, 1 H, H-4^{II}), 4.39 (d, $J_{1,2}=8.0$ Hz, H-1^I), 4.21 (dd, 1 H, $J_{8,9'}=5.2$, $J_{gem}=11.6$ Hz, H-9′^{II}), 4.09 (dd, 1 H, $J_{2,3}=10.0$, $J_{3,4}=3.6$ Hz, H-3^I), 3.99 (dd, 1 H, $J_{8,9}=7.0$, $J_{gem}=11.4$ Hz, H-9^{II}), 3.51 (m, 2 H, $Me_3SiCH_2CH_2$), 2.52 (s, 3 H, AcN), 2.34 (dd, 1 H, $J_{3a,4}=5.7$, $J_{gem}=14.7$ Hz, H-3^{II}α), 2.25 (dd, 1 H, $J_{3\beta,4}=10.2$, $J_{gem}=14.7$ Hz, H-3^{II}β), 2.11, 2.09, 2.07, 2.06, 2.01, 2.008, 2.002 (7s, 21 H, 7 OAc), 0.91 (m, 2 H, $Me_3SiCH_2CH_2$). Anal. Calcd for $C_{36}H_{53}NO_{20}Si$ (847.29): C, 51.00; H, 6.30; N, 1.65. Found: C, 50.89; H, 6.15; N, 1.59.

4.4. 5-Acetylamino-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl 1,5-lactam-(2→3)-2,4,6-tri-*O*-acetyl-α,β-D-galactopyranose (4)

To a solution of **3** (132.1 mg, 0.155 mmol) in CH_2Cl_2 (2 mL) was added CF_3CO_2H (0.56 mL), and the mixture was stirred for 2 h at rt. AcOEt (1 mL) was added, and the mixture was concentrated. Column chromatography (60:1 $CHCl_3$–MeOH) of the residue on silica gel gave **4** (110 mg, 95%) as an amorphous mass; α/β = 3/1. ^{1}H NMR for **4** α ($CDCl_3$): δ 5.82 (dd, 1 H, $J_{6,7}=9.8$, $J_{7,8}=4.1$ Hz, H-7^{II}), 5.61 (d, $J_{1,2}=3.6$, H-1^I), 5.41 (m, 1 H, H-8^{II}),5.05 (dd, 1 H, $J_{1,2}=3.6$, $J_{2,3}=10.5$ Hz, H-2^I), 4.91 (m, 1 H, H-4^{II}, 4.55 (dd, 1 H, $J_{2,3}=10.5$, $J_{3,4}=3.4$ Hz, H-3^I), 2.58 (s, 3 H, AcN), 2.51 (dd, 1 H, $J_{3a,4}=5.2$, $J_{gem}=14.4$ Hz, H-3^{II}α), 2.214, 2.213, 2.13, 2.12, 2.09, 2.07, 2.06 (7 s, 21 H, 7 OAc). Anal. Calcd for $C_{31}H_{41}NO_{20}$ (747.22): C, 49.80; H, 5.53; N, 1.87. Found: C, 49.74; H, 5.33; N, 1.78.

4.5. 5-Acetylamino-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl 1,5-lactam-(2→3)-2,4,6-tri-*O*-acetyl-α-D-galactopyranosyl trichloroacetimidate (5)

To a solution of **4** (110 mg, 0.14mmol) in CH_2Cl_2 (4 mL) was added trichloroacetonitrile (470 μL, 37.6 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 25 μL, 0.14 mmol), and the mixture was stirred for 2 h at 0 °C. The mixture was concentrated, and the residue was chromatographed (100:1 $CHCl_3$–MeOH) on a column of silica gel to give the trichloroacetimidate **5** (123 mg, 94%) as an amorphous mass; $[\alpha]_D$ −10.8° (*c* 0.1, $CHCl_3$) ^{1}H NMR ($CDCl_3$): δ 8.64 (s, 1 H, $NHCCl_3$), 6.47 (d, 1 H, $J_{1,2}=3.8$ Hz, H-1^I), 5.36 (m, 1 H, H-8^{II}), 5.75 (dd, $J_{7,8}=4.1$, $J_{6,7}=9.8$ Hz, H-7^{II}), 5.18 (d, 1 H, H-4^I), 4.85 (m, 1 H, H-4^{II}), 4.21 (dd, 1 H, $J_{8,9'}=5.2$, $J_{gem}=11.6$ Hz, H-9′^{II}), 4.09 (dd, 1 H, $J_{2,3}=10.0$, $J_{3,4}=3.6$ Hz, H-3^I), 3.99 (dd, 1 H, $J_{8,9}=7.0$, $J_{gem}=11.4$ Hz, H-9^{II}), 2.55 (s, 3 H, AcN), 2.42 (dd, 1H, $J_{3a,4}=5.7$, $J_{gem}=14.7$ Hz, H-3^{II}α), 2.34 (dd, 1 H, $J_{3\beta,4}=10.2$, $J_{gem}=14.7$ Hz, H-3^{II}β), 2.18, 2.14, 2.13, 2.05, 2.02, 2.01, 2.00 (7 s, 21 H, 7 OAc). Anal. Calcd for $C_{33}H_{41}Cl_3N_2O_{20}$ (890.13): C, 44.43; H, 4.63; N, 3.14. Found: C, 44.20; H, 4.52; N, 2.86.

4.6. 5-Acetylamino-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl-1,5-lactam-(2→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol (7)

To a solution of **5** (123 mg, 0.13 mmol) and (2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol (**6**; 93 mg, 0.21 mmol) in dry CH_2Cl_2 (1 mL) were added 4 Å MS (type AW300, 1 g), and the mixture was stirred for 4 h at rt, and then cooled to 0 °C. TMSOTf (2.6 μL, 13.8

μmol) was added to the mixture, and this was stirred for 48 h at 0 °C, neutralized with Et_3N and filtered. The combined filtrate and washings was concentrated. Chromatography (100:1 $CHCl_3$–MeOH) of the residue on silica gel afforded **7** (79.2 mg, 49%) as an amorphous mass; $[\alpha]_D$ +16.9° (*c* 0.4, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.07–7.44 (m, 5 H, Ph), 5.93 (dt, 1 H, $J_{4,5}=14.1$, $J_{5,6}=J_{5,6'}=6.6$ Hz, H-5 of sphingosine), 5.75 (dd, $J_{6,7}=9.6$, $J_{7,8}=4.1$ Hz, H-7^{II}), 5.55 (m, 1 H, H-4 of sphingosine), 5.42 (m, 1 H, H-8^{II}), 5.23 (d, 1 H, H-4^{I}), 5.20 (dd, $J_{1,2}=7.7$, $J_{2,3}=10.0$ Hz, H-2^{I}), 4.86 (m, 1H, H-4^{II}), 4.47 (d, $J_{1,2}=8.0$ Hz, H-1^{I}), 3.99 (dd, 1H, $J_{8,9'}=7.0$, $J_{gem}=11.4$ Hz, H-$9'^{II}$), 2.57 (s, 3 H, AcN), 2.38 (dd, 1 H, $J_{3a,4}=5.7$, $J_{gem}=14.5$ Hz, H-$3^{II}\alpha$), 2.31 (dd, 1 H, $J_{3\beta,4}=10.2$, $J_{gem}=14.5$ Hz, H-$3^{II}\beta$), 2.20, 2.16, 2.13, 2.10, 2.09, 2.06, 2.02 (7 s, 21 H, 7 OAc), 1.23 (s, 22 H, 11 CH_2), 0.88 (t, 3 H, $J_{vic}=6.6$ Hz, *Me*CH_2). Anal. Calcd for $C_{56}H_{78}N_4O_{22}$ (1158.51): C, 58.02; H, 6.78; N, 4.83. Found: C, 57.81; H, 6.71; N, 4.78.

4.7. 5-Acetylamino-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl-1,5-lactam-(2→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-3-*O*-benzoyl-2-octadecanamido-4-octadecene-1,3-diol (8)

H_2S was bubbled through a stirred solution of **7** (60.3 mg, 52 μmol) in pyridine (16.6 mL) and water (3.4 mL) for 72 h at 0 °C. The mixture was concentrated, and the residual syrup was treated with octadecanoic acid (46 mg, 0.16 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (WSC; 31 mg, 0.16 mmol) in CH_2Cl_2 (4 mL) 12 h at rt. The mixture was extracted with $CHCl_3$, and the extract was successively washed with 1 M HCl and water, dried (Na_2SO_4) and concentrated. Column chromatography (80:1 $CHCl_3$–MeOH) of the residue on silica gel gave **8** (27.3 mg, 38%) as an amorphous mass; $[\alpha]_D$ +12.3° (*c* 0.2, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.04–7.42 (m, 5 H, Ph), 5.97 (br-d, 1 H, NH of sphingosine), 5.87 (dt, 1 H, $J_{4,5}=15.0$, $J_{5,6}=J_{5,6'}=7.0$ Hz, H-5 of sphingosine), 5.77 (dd, $J_{6,7}=9.8$, $J_{7,8}=4.1$ Hz, H-7^{II}), 5.50 (m, 1 H, H-4 of sphingosine), 5.42 (m, 1 H, H-8^{II}), 5.20 (d, 1H, H-4^{I}), 5.12 (dd, 1H, $J_{1,2}=7.3$, $J_{2,3}=9.6$ Hz, H-2^{I}), 4.87 (m, 1H, H-4^{II}), 4.40 (d, $J_{1,2}=7.3$ Hz, H-1^{I}), 4.25 (dd, 1H, $J_{8,9}=5.2$, $J_{gem}=11.8$ Hz, H-9^{II}), 4.06 (dd, 1 H, $J_{2,3}=9.8$, $J_{3,4}=3.4$ Hz, H-3^{I}), 2.57 (s, 3 H, AcN), 2.38 (dd, 1H, $J_{3a,4}=5.7$, $J_{gem}=14.6$ Hz, H-$3^{II}\alpha$), 2.35 (dd, 1H, $J_{3\beta,4}=10.5$, $J_{gem}=14.6$ Hz, H-$3^{II}\beta$), 2.19, 2.17, 2.13, 2.10, 2.06, 2.03, 1.85 (7 s, 21 H, 7 OAc), 1.26 (s, 52 H, 26 CH_2), 0.88 (t, 6 H, $J_{vic}=6.4$ Hz, 2 *Me*CH_2). Anal. Calcd for $C_{74}H_{114}N_2O_{23}$ (1398.71): C, 63.50; H, 8.21; N, 2.00. Found: C, 63.37; H, 8.07; N, 1.85.

4.8. 5-Amino-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl 1,5-lactam-(2→3)-β-D-galactopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-2-octadecanamido-4-octadecene-1,3-diol (9, GSC-538)

To a solution of **8** (11.7 mg, 8.3 μmol) in MeOH (4 mL) and dioxane (0.4 mL) was added a catalytic amount of 28% NaOMe in MeOH, and the mixture was stirred for 72 h at rt. The mixture was neutralized with Amberlite IR-120 (H) resin and filtered. The resin was washed with MeOH, and the combined filtrate and washings was concentrated. Column chromatography (1:1 $CHCl_3$–MeOH) of the residue on Sephadex LH-20 gave the target molecule (8 mg, quant) as an amorphous mass; $[\alpha]_D$ −10.6° (*c* 0.6, 1:1 $CHCl_3$–MeOH); ^1H NMR (CD_3OD): δ 5.58 (dt, 1 H, $J_{4,5}=14.5$, $J_{5,6}=J_{5,6'}=6.8$ Hz, H-5 of sphingosine). 5.32 (m, 1 H, H-4 of sphingosine), 4.19 (d, $J_{1,2}=7.7$ Hz, H-1^{I}), 3.94 (dd, 1 H, $J_{2,3}=9.8$, $J_{3,4}=3.2$ Hz, H-3^{I}), 2.30 (dd, 1H, $J_{3\beta,4}=10.5$, $J_{gem}=13.9$ Hz, H-$3^{II}\beta$), 2.07 (t, 2 H, $J_{gem}=14.8$ Hz, H-1′ of stearoyl), 2.02 (dd, 1 H, $J_{3a,4}=4.8$, $J_{gem}=13.9$ Hz, H-3^{II} α),1.47 (m, 1 H, of stearoyl), 1.22 (s, 52 H, 26 CH_2), 0.88 (t, 6 H, $J_{vic}=7.5$ Hz, 2 *Me*CH_2). Anal. Calcd for $C_{51}H_{94}N_2O_{14}$ (958.67): C, 63.85; H, 9.88; N, 2.92. Found: C, 63.66; H, 9.80; N, 2.87.

4.9. 2-(Trimethylsilyl)ethyl 2-acetamido-2-deoxy-3-*O*-4-methyoxybenzyl-6-*O*-4-methoxyphenyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (11)

To a solution of **10** (527 mg, 0.403 mmol) in THF (8 mL) were added PPh_3 (522 mg, 1.98 mmol), DEAD (633 μL, 1.44 mmol), and MPOH (300 mg, 2.40 mmol), and the mixture was stirred under reflux for 12 h. After completion of the reaction, the mixture was concentrated. Column chromatography (1:1 AcOEt–hexane) of the residue on silica gel afforded **11** (430 mg, 76%) as an amorphous mass; $[\alpha]_D$ −2.6° (*c* 1.63, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.35–6.73 (m, 38 H, 2 MeO*Ph*, 6 Ph). 4.41 (d, 1 H, $J_{1,2}=7.8$ Hz, H-1^{II}), 4.33 (d, 1 H, $J_{1,2}=7.6$ Hz, H-1^{III}), 4.09 (dd, 1 H, $J_{2,3}=10.1$, $J_{3,4}=4.1$ Hz, H-3^{II}), 3.98 (dd, 1 H, H-$6'^{III}$), 3.93 (dd, 1 H, $J_{5,6}=5.7$, $J_{gem}=12.3$ Hz, H-6^{III}), 3.83–3.91 (m, 2 H, H-4^{III} and H-5^{II}), 3.68, 3.73 (2 s, 6 H, 2 *Me*OPh), 3.71 (d, 1 H, H-4^{II}), 3.67 (dd, 1 H, H-2^{II}), 3.57–3.61 (m, 3 H, $Me_3SiCH_2CH_2$ and H-5^{III}), 3.50 (t, 1 H, $J_{3,4}=9.6$ Hz, H-3^{III}), 3.35 (dd, 1 H, $J_{2,3}=9.2$ Hz, H-2^{III}), 3.33 (dd, 1 H, $J_{5,6}=4.1$, $J_{gem}=12.5$ Hz, H-6^{II}), 1.45 (s, 3 H, AcN), 1.00 (m, 2 H, $Me_3SiCH_2CH_2$). ^{13}C NMR ($CDCl_3$): δ 171.53 (C=O), 160.80, 155.49, 155.42 (MeO*Ph*), 140.76, 140.60, 140.50, 140.20, 139.73, 139.67, 131.70, 131.03, 129.95, 129.74, 129.66, 129.64, 129.55, 129.40, 129.34, 129.13, 129.04, 128.97, 128.88, 128.86, 128.67, 128.48, 127.90 (arom-C), 117.15, 116.07, 115.44 (MeO*Ph*),

104.50, 103.97, 103.06, 84.26, 83.23, 83.11, 82.66, 81.64, 78.02, 77.52, 76.73, 76.51, 76.34, 76.13, 75.89, 75.44, 74.82, 74.66, 72.52, 70.04, 69.72, 69.67, 68.70, 57.51, 57.09, 56.65, 24.51, 19.86. Anal. Calcd for $C_{82}H_{97}NO_{18}Si$ (1411.65): C, 69.71; H, 6.92; N, 0.99; found: C, 69.48; H, 6.69; N, 0.76.

4.10. 2-(Trimethylsilyl)ethyl methyl 4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-trifluoroacetamido-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-3-*O*-4-methyoxybenzyl-6-*O*-4-methoxyphenyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (13)

To a solution of **12** (477 mg, 0.43 mmol) and the trisaccharide acceptor **11** (430 mg, 0.30 mmol) in dry CH_2Cl_2 (4.5 mL) were added 4 Å MS (450 mg), and the mixture was stirred for 3 h at rt, then cooled to 0 °C. TMSOTf (10.75 μL, 53.7 μmol) was added to the mixture that was stirred for 18 h at 2 °C, neutralized with Et_3N and filtered. The residue was washed with $CHCl_3$. The combined filtrate and washings was concentrated. Column chromatography (80:1 $CHCl_3$–MeOH) of the residue on silica gel gave **13** (599 mg, 81.3%) as an amorphous mass; $[\alpha]_D$ +32.8° (*c* 6.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.15–6.58 (m, 53 H, 2 MeO*Ph*, 9 Ph). 6.49 (d, 1 H, $J_{5,NH}$ = 8.9 Hz, NH^V), 5.53 (m, 1 H, H-8^V), 5.48 (dd, 1 H, $J_{1,2}$ = 7.8, $J_{2,3}$ = 10.1 Hz, H-2^{IV}), 5.39 (d, 1H, H-4^{IV}), 5.34 (d, 1 H, $J_{2,NH}$ = 8.9 Hz, NH^{III}), 5.20 (dd, 1 H, H-7^V), 5.10 (d, 1 H, $J_{1,2}$ = 7.8 Hz, H-1^{IV}), 4.94 (dd, 1 H, $J_{2,3}$ = 10.0, $J_{3,4}$ = 3.2 Hz, H-3^{IV}), 4.59 (dd, 1 H, $J_{1,2}$ = 7.1, $J_{2,3}$ = 10.2 Hz, H-2^I), 4.25 (d, 1 H, $J_{1,2}$ = 7.3 Hz, H-1^{III}), 4.23 (d, 1 H, $J_{1,2}$ = 8.2 Hz, H-1^{II}), 4.17 (d, 1 H, $J_{1,2}$ = 7.1 Hz, H-1^I), 3.96 (dd, 1 H, $J_{1,2}$ = 7.3, $J_{2,3}$ = 10.7 Hz, H-2^{III}), 3.89 (dd, 1 H, $J_{8,9}$ = 5.7, J_{gem} = 13.5 Hz, H-9^V), 3.81 (s, 3 H, COO*Me*), 3.68, 3.63 (2 s, 6 H, 2 *Me*OPh), 3.51 (m, 2 H, $Me_3SiCH_2CH_2$), 2.50 (dd, 1 H, $J_{3eq,4}$ = 4.3, J_{gem} = 12.5 Hz, H-3^Veq), 2.14, 1.93, 1.87, 1.48 (4 s, 12 H, 4 AcO), 1.65 (t, 1 H, $J_{gem} = J_{3ax,4}$ = 12.5 Hz, H-3^Vax), 1.45 (s, 3 H, AcN), 1.01 (m, 2 H, $Me_3SiCH_2CH_2$). ^{13}C NMR ($CDCl_3$): δ 172.17 (C=O), 172.14 (C=O), 172.08 (C=O), 171.40 (C=O), 171.14 (C=O), 169.27 (C=O), 167.24 (C=O), 167.17 (C=O), 167.00 (C=O), 160.26, 155.35, 154.09 (MeO*Ph*), 140.71, 140.59, 140.55, 140.17, 139.82, 139.73, 134.95, 134.82, 134.58, 131.86, 131.53, 131.28, 131.06, 130.96, 130.62, 130.56, 130.15, 129.93, 129.73, 129.64, 129.61, 129.55, 129.49, 129.45, 129.38, 129.32, 129.10, 128.99, 128.95, 128.87, 128.83, 128.77, 128.68, 128.42, 128.01 (arom-C), 116.86, 115.96, 114.91 (MeO*Ph*), 104.45, 103.85, 103.78, 101.40, 98.32, 84.34, 84.08, 83.33, 80.93, 80.91, 77.36, 76.67, 76.54, 76.31, 76.05, 75.86, 74.67, 74.58, 73.93, 73.15, 72.80, 72.63, 72.04, 70.32, 69.88, 69.72, 69.56, 69.49, 69.12, 68.68, 67.72, 63.35, 57.02, 56.84, 55.36, 54.73, 51.02, 38.68, 24.26, 22.75, 21.99, 21.79, 21.49, 19.85. Anal. Calcd for $C_{129}H_{143}F_3N_2O_{38}Si$ (2412.90): C, 64.17; H, 5.97; N, 1.16. Found: C, 64.09; H, 5.71; N, 1.09.

4.11. 2-(Trimethylsilyl)ethyl methyl 4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-trifluoroacetamido-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2-acetamido-3-*O*-acetyl-2-deoxy-6-*O*-4-methoxyphenyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (14)

A solution of **13** (284 mg, 0.11 mmol) in EtOH (20 mL) and HOAc (4 mL) was vigorously stirred with $Pd(OH)_2$ (285 mg) for 48 h at rt under hydrogen. The catalyst was collected and washed with MeOH (Caution! Extreme fire hazard). The combined filtrate and washings was concentrated, and the residue was treated with Ac_2O (5 mL) and pyridine (8 mL) for 12 h at rt, then cooled to 0 °C. MeOH (3 mL) was added and the mixture was concentrated, and the residue was extracted with $CHCl_3$ and successively washed with cold 2 M HCl and water, dried (Na_2SO_4) and concentrated. Column chromatography (50:1 $CHCl_3$–MeOH) of the residue on silica gel gave **14** (215 mg, 90%) as an amorphous mass; $[\alpha]_D$ + 17.1° (*c* 0.7, $CHCl_3$) ^{1}H NMR ($CDCl_3$): δ 8.12–6.47 (m, 19 H, MeOPh, 3 Ph), 6.18 (d, 1 H, $J_{NH,2}$ = 8.9 Hz, NH^{III}), 5.56 (m, 1 H, H-8^V), 5.39 (d, 1 H, $J_{NH,5}$ = 8.7 Hz, NH^V), 5.36 (dd, 1 H, $J_{1,2}$ = 7.8, $J_{2,3}$ = 10.0 Hz, H-2^{IV}), 5.34 (d, 1 H, H-4^{II}), 5.16 (dd, 1 H, $J_{6,7}$ = 2.5, $J_{7,8}$ = 9.4 Hz, H-7^V), 5.13 (t, 1 H, $J_{2,3}$ = 9.4 Hz, H-3^I), 5.06 (d, 1 H, $J_{1,2}$ = 8.0 Hz, H-1^{IV}), 4.98 (dd, 1 H, $J_{1,2}$ = 8.0, $J_{2,3}$ = 9.8 Hz, H-2^I), 4.85 (dd, 1 H, $J_{1,2}$ = 8.0, $J_{2,3}$ = 9.6 Hz, H-2^{II}), 4.82 (dd, 1 H, $J_{2,3}$ = 10.0, $J_{3,4}$ = 3.2 Hz, H-3^{IV}), 4.54 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1^{III}),4.46 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1^{II}), 4.28 (d, 1 H, $J_{1,2}$ = 8.0 Hz, H-1^I), 3.80 (s, 3 H, COO*Me*), 3.75 (s, 3 H, *Me*OPh), 3.54 (m, 2 H, $Me_3SiCH_2CH_2$), 2.51 (dd, 1 H, $J_{3eq,4}$ = 4.6, J_{gem} = 12.5 Hz, H-3^Veq), 2.13, 2.10, 2.06, 2.05, 2.04, 2.03, 2.01, 1.98, 1.93, 1.90, 1.89 (11 s, 33 H, 11 AcO), 1.59 (t, 1H, $J_{gem} = J_{3ax,4}$ = 12.5 Hz, H-3^Vax), 1.42 (s, 3 H, AcN), 0.88 (m, 2 H, $Me_3SiCH_2CH_2$). ^{13}C NMR ($CDCl_3$): δ = 171.16 (C=O), 170.76 (C=O), 170.72 (C=O), 170.65 (C=O), 170.54 (C=O), 170.49 (C=O), 170.44 (C=O), 170.38 (C=O), 170.11 (C=O), 169.98 (C=O), 169.94 (C=O), 169.76 (C=O), 169.61 (C=O), 169.24 (C=O), 167.96 (C=O), 165.23 (C=O), 154.03, 153.00 (MeO*Ph*), 135.05, 134.86, 133.37, 131.66, 131.30, 131.06, 130.73, 130.52, 130.24, 130.02, 129.71, 128.68 (arom-C), 115.69, 114.67 (MeO*Ph*), 102.64, 102.21, 101.32, 100.73, 96.73, 76.43, 74.07, 73.58, 72.16, 71.77, 71.73, 71.28, 71.21, 71.07, 70.96, 70.80, 69.94, 69.00, 68.45, 67.59, 67.50, 67.35, 66.34, 64.73, 62.23, 61.89, 61.96, 55.68, 53.22, 48.43, 37.20, 29.71, 23.72, 23.51, 21.44, 21.05, 20.86, 20.78,

20.69, 20.52, 20.35, 19.68. Anal. Calcd for $C_{93}H_{113}F_3N_2O_{44}Si$ (2046.64): C, 54.54; H, 5.56; N, 1.37. Found: C, 54.54; H, 5.39; N, 1.17.

4.12. 2-(Trimethylsilyl)ethyl methyl 4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-trifluoroacetamido-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2-acetamido-3-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (15)

To a solution of **14** (215 mg, 0.105 mmol) in MeCN (8.1 mL) and water (0.9 mL) was added CAN (205 mg, 0.35 mmol), and the mixture was stirred for 45 min at 0 °C and extracted with AcOEt. The extract was successively washed with M Na_2CO_3 and water, dried (Na_2SO_4) and concentrated. Column chromatography (50:1 $CHCl_3$–MeOH) of the residue on silica gel gave **15** (203 mg, 99.6%) as an amorphous mass; $[\alpha]_D$ +4.9° (*c* 1.8, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.22–7.42 (m, 15 H, 3 Ph), 5.68 (m, 1 H, H-8^V),5.41 (d, 1 H, H-4^{IV}), 5.36 (dd, 1 H, $J_{1,2}=8.0$, $J_{2,3}=10.1$ Hz, H-2^{IV}), 4.96 (dd, 1 H, $J_{1,2}=8.0$, $J_{2,3}=9.8$ Hz, H-2^I), 4.85 (dd, 1 H, $J_{1,2}=8.0$, $J_{2,3}=9.6$ Hz, H-2^{II}), 4.60 (d, 1 H, $J_{1,2}=8.0$ Hz, H-1^{III}), 4.45 (d, 1 H, $J_{1,2}=8.0$ Hz, H-1^{II}), 4.31 (d, 1 H, $J_{1,2}=8.0$ Hz, H-1^I), 4.09 (dd, 1 H, $J_{8,9'}=5.5$, $J_{gem}=12.1$ Hz, H-9'^{V}), 3.82 (s, 3 H, COO*Me*), 3.60 (dd, 1 H, $J_{1,2}=8.0$, $J_{2,3}=10.1$ Hz, H-2^{III}), 3.52 (m, 2 H, $Me_3SiCH_2CH_2$), 3.23 (br-d, H-6^{III}), 2.49 (dd, 1 H, $J_{3eq,4}=4.6$, $J_{gem}=12.5$ Hz, H-3^V*eq*), 2.16, 2.11, 2.10, 2.09, 2.06, 2.03, 2.01, 1.99, 1.96, 1.89, 1.87 (11 s, 33 H, 11 AcO), 1.62 (t, 1 H, $J_{gem}=J_{3ax,4}=12.5$ Hz, H-3^V*ax*), 1.46 (s, 3 H, AcN^{III}), 0.87 (m, 2 H, $Me_3SiCH_2CH_2$).

^{13}C NMR ($CDCl_3$): δ 173.31 (C=O), 172.20 (C=O), 172.11 (C=O), 172.04 (C=O), 172.03 (C=O), 171.86 (C=O), 171.67 (C=O), 171.59 (C=O), 171.45 (C=O), 171.38 (C=O), 171.22 (C=O), 171.13 (C=O), 170.45 (C=O), 169.57 (C=O), 167.21 (C=O), 166.85 (C=O), 134.67, 134.41, 134.65, 131.76, 131.66, 130.99, 130.64, 130.02, 129.67 (arom-C), 102.11, 102.03, 101.56, 101.26, 98.24, 77.32, 76.88, 76.53, 74.30, 74.11, 74.03, 73.52, 73.17, 72.88, 72.65, 72.54, 71.67, 70.81, 70.65, 69.34, 68.78, 68.79, 67.83, 63.67, 63.64, 63.55, 62.99, 62.30, 56.24, 54.55, 50.19, 39.11, 38.62, 31.38, 29.20, 24.66, 22.87, 22.31, 22.24, 22.25, 22.14, 21.83, 19.28. Anal. Calcd for $C_{86}H_{107}F_3N_2O_{43}Si$ (1940.60): C, 53.19; H, 5.55; N, 1.44. Found: C, 52.95; H, 5.48; N, 1.17.

4.13. 2-(Trimethylsilyl)ethyl methyl 4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-trifluoroacetamido-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2-acetamido-3-*O*-acetyl-2-deoxy-6-*O*-levulinoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (16)

To a solution of **15** (186.2 mg, 95.9 μmol) in pyridine (6 mL) was added levulinic anhydride (50 mg, 0.23 mmol) and DMAP (20 mg, 0.16 mmol), and the mixture was stirred for 48 h at 65 °C, then cooled to 0 °C. MeOH (3 mL) was added and the mixture was concentrated, and the residue was extracted with $CHCl_3$ and successively washed with cold 2 M HCl and water, dried (Na_2SO_4) and concentrated. Column chromatography (50:1 $CHCl_3$–MeOH) of the residue on silica gel gave **16** (140.4 mg, 73%) as an amorphous mass; $[\alpha]_D$ +8.6° (*c* 1.6, $CHCl_3$), ^{1}H NMR ($CDCl_3$): δ 8.08–7.45 (m, 15 H, 3 Ph), 6.13 (d, 1 H, $J_{NH,2}=9.8$ Hz, NH^{III}), 5.63 (m, 1 H, H-8^V), 5.39–5.36 (m, 2 H, H-2^{IV} and H-4^{IV}), 5.29 (d, 1 H, $J_{5,NH}=8.7$ Hz, NH^V), 5.26 (d, 1 H, H-4^{II}), 5.21 (dd, 1 H, $J_{6,7}=2.3$, $J_{7,8}=9.4$ Hz, H-7^V), 5.15 (dd, 1 H, H-3^{II}), 4.98 (dd, 1 H, $J_{1,2}=8.1$, $J_{2,3}=9.8$ Hz, H-2^I), 4.92 (d, 1 H, $J_{1,2}=7.8$ Hz, H-1^{IV}), 4.87 (dd, 1 H, $J_{1,2}=8.0$, $J_{2,3}=9.8$ Hz, H-2^{II}), 4.83 (dd, 1 H, $J_{3,4}=3.2$ Hz, H-3^{IV}), 4.55 (d, 1 H, $J_{1,2}=8.2$ Hz, H-1^{III}), 4.46 (d, 1H, $J_{1,2}=8.0$ Hz, H-1^{II}), 4.36 (d, 1H, $J_{1,2}=8.0$ Hz, H-1^I), 4.04–4.01 (m, 2 H, H-9'^{V} and H-9^V), 3.82 (s, 3 H, COO*Me*), 3.59 (m, 2 H, $Me_3SiCH_2CH_2$), 3.55 (dd, 1 H, $J_{2,3}=10.1$ Hz, H-2^{III}), 2.71–2.44 (m, 5 H, MeCOCH_2CH_2 and H-3^V*eq*), 2.154, 2.151, 2.10, 2.09, 2.08, 2.06, 2.04, 2.02, 2.01, 1.99, 1.90, 1.89 (12 s, 36 H, 11 AcO and *Me*COCH$_2$CH$_2$), 1.61 (t, 1H, $J_{gem}=J_{3ax,4}=12.5$ Hz, H-3^V*ax*), 1.49 (s, 3 H, AcN^{III}), 0.93 (m, 2 H, $Me_3SiCH_2CH_2$).

^{13}C NMR ($CDCl_3$): δ 208.3 (C=O), 172.73 (C=O), 172.16 (2C=O), 171.89 (2C=O), 171.45 (2C=O), 171.22 (2C=O), 171.04 (2C=O), 170.11 (C=O), 169.33 (C=O), 167.19 (C=O), 166.93 (2C=O), 166.34 (C=O), 134.86, 134.73, 131.72, 131.40, 131.18, 130.65, 130.28, 130.03, 129.97, 129.84 (arom-C), 102.69, 102.18 (2C) 101.39, 98.25, 77.37, 76.57, 75.65, 74.22, 74.00, 73.56, 73.12, 72.79, 72.41, 72.30, 72.11, 71.95, 70.44, 69.97, 69.40, 68.92, 68.47, 68.16, 64.29, 63.59, 63.04, 62.88, 61.80, 56.23, 54.72, 50.88, 38.64, 31.13, 24.67, 22.82, 22.23, 22.18, 22.06, 21.83, 21.57, 19.29. Anal. Calcd for $C_{91}H_{113}F_3N_2O_{45}Si$ (2038.63): C, 53.58; H, 5.58; N, 1.37. Found: C, 53.47; H, 5.47; N, 1.12.

4.14. Methyl 4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-trifluoroacetamido-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2-acetamido-3-*O*-acetyl-2-deoxy-6-*O*-levulinoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate (17)

The 2-(trimethylsilyl)ethyl group of **16** (140.4 mg, 68.8 μmol) was removed by treatment with CF_3CO_2H (1.4 mL) in CH_2Cl_2 (4 mL) for 3 h at rt. AcOEt (2 mL) was added, and the mixture was concentrated. Column chromatography (20:1 $CHCl_3$–MeOH) of the residue on silica gel gave the 1-OH free derivative (134.2 mg quant). This compound was treated with trichloroacetonitrile (188 μL, 15.0 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU; 9.8 μL, 60 μmol) in CH_2Cl_2 (4 mL) for 2 h at 0 °C. The mixture was concentrated, and the residue was chromatographed (30:1 $CHCl_3$–MeOH) on a column of silica gel to give the trichloroacetimidate **17** (135.3 mg, 95%) as an amorphous mass; $[\alpha]_D$ +5.2° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.65 (s, 1 H, NH of imidate), 8.12–7.36 (m, 15 H, 3 Ph), 6.45 (d, 1 H, $J_{1,2}=3.7$ Hz, H^{I}), 5.63 (m, 1 H, H-8^{V}), 5.49 (t, 1 H, $J_{2,3}=9.6$ H-3^{I}), 5.39 (dd, 1 H, $J_{1,2}=8.5$, $J_{2,3}=10.3$ Hz, H-2^{IV}), 5.21 (dd, 1 H, $J_{6,7}=2.3$, $J_{7,8}=9.4$ Hz, H-7^{V}), 5.04 (dd, 1 H, $J_{1,2}=3.7$, $J_{2,3}=10.1$ Hz, H-2^{I}), 4.92 (d, 1 H, $J_{1,2}=7.8$ Hz, H-1^{IV}), 4.87 (dd, 1 H, $J_{1,2}=8.0$, $J_{2,3}=9.8$ Hz, H-2^{II}), 4.51 (d, 1 H, $J_{1,2}=8.0$ Hz, H-1^{III}), 4.31 (d, 1H, $J_{1,2}=8.0$ Hz, H-1^{II}), 3.82 (s, 3 H, COO*Me*), 2.75–2.44 (m, 5 H, MeCO*CH*$_2$*CH*$_2$ and H-3^{V}*eq*), 2.154, 2.151, 2.10, 2.09, 2.08, 2.06, 2.04, 2.02, 2.01, 1.99, 1.90, 1.89 (12 s, 36 H, 11 AcO and *Me*COCH$_2$CH$_2$), 1.60 (t, 1H, $J_{gem}=J_{3ax,4}=$ 12.5 Hz, H-3^{V}*ax*), 1.42 (s, 3 H, AcN^{III}). Anal. Calcd for $C_{88}H_{101}Cl_3F_3N_3O_{45}$ (2081.47): C, 50.71; H, 4.88; N, 2.02. Found: C, 50.52; H, 4.65; N, 1.78.

4.15. Methyl 4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-trifluoroacetamido-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2-acetamido-3-*O*-acetyl-2-deoxy-6-*O*-levulinoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol (18)

To a solution of **17** (135.3 mg, 65 μmol) and **6** (42 mg, 97 μmol) in dry CH_2Cl_2 (0.7 mL) were added 4 Å MS (type AW300, 500 mg), and the mixture was stirred for 6 h at rt, and then cooled to 0 °C. TMSOTf (1.17 μL, 5.98 μmol) was added to the mixture, and this was stirred for 48 h at 0 °C, neutralized with Et_3N and filtered. Chromatography (60:1 $CHCl_3$–MeOH) of the residue on silica gel afforded **18** (31.7 mg, 20.7%) as an amorphous mass; $[\alpha]_D$ +8.3° (*c* 0.64, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.18–7.43 (m, 20 H, 4 Ph), 5.99 (d, 1 H, $J_{NH,2}=9.6$ Hz, NH^{III}), 5.90 (dt, 1 H, $J_{4,5}=14.8$, $J_{5,6}=J_{5,6'}=6.8$ Hz, H-5 of sphingosine), 5.65 (m, 1 H, H-8^{V}), 5.59 (m, 1 H, H-4 of sphingosine), 5.37 (dd, 1 H, $J_{1,2}=7.6$, $J_{2,3}=10.1$ Hz, H-2^{IV}), 5.35 (d, 1 H, H-4^{IV}), 5.20 (dd, 1 H, $J_{6,7}=2.7$, $J_{7,8}=9.8$ Hz, H-7^{V}), 5.16 (dd, 1 H, H-2^{I}), 4.97 (dd, 1 H, $J_{1,2}=7.8$, $J_{2,3}=10.1$ Hz, H-2^{II}), 4.91 (d, 1 H, $J_{1,2}=7.6$ Hz, H-2^{IV}), 4.83 (dd, 1 H, $J_{2,3}=$ 10.1, $J_{3,4}=3.2$ Hz, H-3^{IV}), 4.54 (d, 1 H, $J_{1,2}=7.8$ Hz, H-1^{III}), 4.49 (d, 1 H, $J_{1,2}=7.8$ Hz, H-1^{II}), 4.35 (d, 1 H, $J_{1,2}=8.2$ Hz, H-1^{I}), 3.82 (s, 3 H, COO*Me*), 3.67 (dd, 1 H, $J_{2,3}=10.1$ Hz, H-2^{III}), 2.70–2.43 (m, 5 H, MeCO*CH*$_2$*CH*$_2$ and H-3^{V}*eq*), 2.149, 2.148, 2.10, 2.08, 2.065, 2.061, 2.03, 2.01, 2.00, 1.99, 1.89, 1.88 (12 s, 36 H, 11 AcO and *Me*COCH$_2$CH$_2$), 1.60 (t, 1 H, $J_{gem}=$ $J_{3ax,4}=12.5$ Hz, H-3^{V}*ax*), 1.48 (s, 3 H, AcN^{III}), 1.23 (s, 22 H, 11 CH_2), 0.88 (t, 3 H, $J_{vic}=6.9$ Hz, 2 *Me*CH$_2$). ^{13}C NMR ($CDCl_3$): δ 171.98 (C=O), 170.81 (C=O), 170.66 (C=O), 170.54 (C=O), 170.21 (C=O), 170.07 (C=O), 169.84 (C=O), 169.81 (C=O), 169.58 (C=O), 169.56 (C=O), 169.00 (C=O), 168.53 (C=O), 167.90 (C=O), 165.79 (C=O), 165.51 (C=O), 165.11 (C=O), 163.60 (C=O), 133.25, 131.61, 130.32, 129.92, 129.77, 129.58, 129.22, 129.00, 128.64, 128.49, 128.83, 125.61, 124.35, 122.60, 122.55 (arom-C), 100.85, 100.74, 100.47, 100.38, 96.87, 76.39, 75.62, 75.33, 74.70, 73.80, 72.85, 72.63, 72.47, 72.11, 71.94, 71.67, 71.59, 71.38, 70.99, 70.92, 70.55, 69.55, 68.39, 68.20, 67.85, 67.48, 67.28, 66.56, 66.25, 63.54, 62.02, 61.26, 53.40, 53.27, 49.74, 37.77, 37.15, 32.41, 31.95, 29.91, 29.87, 29.75, 29.68, 29.61, 29.42, 29.39, 29.19, 28.75, 27.81, 23.82, 23.25, 22.73, 22.01, 21.42, 20.92, 20.77, 20.43, 20.19, 19.64, 14.17. Anal. Calcd for $C_{111}H_{138}F_3N_5O_{47}$ (2349.85): C, 56.70; H, 5.92; N, 2.98. Found: C, 56.41; H, 5.82; N, 2.75.

4.16. Methyl 4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-trifluoroacetamido-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2-acetamido-3-*O*-acetyl-2-deoxy-6-*O*-levulinoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-3-*O*-benzoyl-2-octadecanamido-4-octadecene-1,3-diol (19)

H_2S was bubbled through a stirred solution of **18** (31.7 mg, 13.5 μmol) in pyridine (5 mL) and water (1 mL) for 72 h at 0 °C. The mixture was concentrated and the residual syrup was treated with octadecanoic acid (11.5 mg, 0.04 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (WSC; 7.7 mg, 40 μmol) in CH_2Cl_2 (1 mL) for 24 h at rt. The mixture was extracted with $CHCl_3$, and the extract was successively washed with M HCl and water, dried (Na_2SO_4) and concentrated. Column chromatography (50:1 $CHCl_3$–MeOH) of the residue on silica gel gave **19** (23 mg, 66%) as an amorphous mass; $[\alpha]_D$ +3.5° (*c* 0.25, $CHCl_3$); ^{1}H NMR

($CDCl_3$): δ 8.22–7.42 (m, 20 H, 4 Ph), 6.00 (d, 1 H, $J_{NH,2} = 9.8$ Hz, NH^{III}), 5.85 (dt, 1 H, $J_{4,5} = 14.6$, $J_{5,6} = J_{5,6'} = 6.6$ Hz, H-5 of sphingosine), 5.73 (d, 1 H, $J_{NH,2} = 8.9$ Hz, NH of sphingosine), 5.64 (m, 1 H, H-8^{V}), 5.45 (m, 1 H, H-4 of sphingosine), 5.38–5.35 (m, 2 H, H-4^{IV} and H-2^{IV}), 5.21 (dd, 1 H, $J_{6,7} = 2.3$, $J_{7,8} = 10.1$ Hz, H-7^{V}), 4.95 (d, 1 H, $J_{1,2} = 8.9$ Hz, H-1^{IV}), 4.91 (dd, 1 H, $J_{1,2} = 7.6$, $J_{2,3} = 9.8$ Hz, H-2^{I}), 4.87 (dd, 1 H, $J_{1,2} = 7.8$, $J_{2,3} = 9.2$ Hz, H-2^{II}), 4.83 (dd, 1 H, $J_{2,3} = 10.7$, $J_{3,4} = 2.7$ Hz, H-3^{IV}), 4.53 (d, 1 H, $J_{1,2} = 8.2$ Hz, H-1^{III}), 4.42 (d, 1 H, $J_{1,2} = 7.8$ Hz, H-1^{II}), 4.31 (d, 1 H, $J_{1,2} = 7.6$ Hz, H-1^{I}), 4.03 (dd, 1 H, $J_{8,9'} = 4.8$, $J_{gem} = 11.5$ Hz, H-$9'^{V}$), 3.94 (dd, 1 H, $J_{8,9} = 5.5$, $J_{gem} = 11.5$ Hz, H-9^{V}), 3.82 (s, 3 H, COO*Me*), 3.67 (t, 1 H, $J_{2,3} = 9.8$ Hz, H-2^{III}), 2.70–2.43 (m, 5 H, MeCOCH_2CH_2 and H-$3^{V}eq$), 2.14, 2.12, 2.11, 2.09, 2.08, 2.06, 2.009, 2.007, 1.99, 1.91. 1.89, 1.88 (12 s, 36 H, 11 AcO and *Me*$COCH_2CH_2$), 1.60 (t, 1 H, $J_{gem} = J_{3ax,4} = 12.5$ Hz, H-$3^{V}ax$), 1.48 (s, 3 H, AcN^{III}), 1.24 (s, 52 H, 26 CH_2), 0.87 (t, 6 H, $J_{vic} = 6.2$ Hz, 2 *Me*CH_2). ^{13}C NMR ($CDCl_3$): δ 172.63 (C=O), 171.95 (C=O), 170.76 (C=O), 170.61 (C=O), 170.47 (C=O), 170.43 (C=O), 170.31 (C=O), 170.15 (C=O), 169.80 (C=O), 169.69(C=O), 169.57 (C=O), 168.93 (C=O), 167.85 (C=O), 165.75 (C=O), 165.46 (C=O), 165.17 (C=O), 164.80 (C=O), 164.05 (C=O), 133.45, 133.35, 133.04, 130.28, 130.22, 129.99, 129.74, 129.58, 129.44, 128.61, 128.44, 128.39 (arom-C), 100.47, 100.63, 100.48, 100.35, 96.18, 76.40, 75.44, 75.08, 74.68, 74.03, 72.80, 72.27, 72.04, 71.64, 71.25, 71.16, 70.94, 70.51, 69.15, 68.46, 67.81, 67.40, 67.17, 66.35, 66.20, 61.99. 61.46, 61.30, 54.87, 53.33, 50.57, 49.68, 37.74, 37.10, 36.85, 32.33, 31.92, 29.83, 29.74, 29.63, 29.53, 29.49, 29.44, 29.36, 29.25, 28.94, 27.77, 25.73, 23.22, 22.69, 21.39, 20.86, 20.74, 20.58, 20.39, 20.15, 14.13. Anal. Calcd for $C_{129}H_{174}F_3N_3O_{48}$ (2590.12): C, 59.78; H, 6.77; N, 1.62. Found: C, 59.64; H, 6.54; N, 1.59.

4.17. Methyl 4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-trifluoroacetamido-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2-acetamido-3-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-3-*O*-benzoyl-2-octadecanamido-4-octadecene-1,3-diol (20)

To a solution of **19** (23 mg, 8.8 μmol) in EtOH (1 mL) was added hydrazine acetate (4.2 mg, 45 μmol), and the mixture was stirred for 4 h at rt and then concentrated. Column chromatography (50:1 $CHCl_3$–MeOH) of the residue on silica gel gave **20** (16.2 mg, 73%) as an amorphous mass; $[\alpha]_D$ −13.3° (*c* 0.032, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.22–7.42 (m, 20 H, 4 Ph), 5.99 (d, 1 H, $J_{NH,2} = 8.9$ Hz, NH^{III}), 5.87 (dt, 1 H, $J_{4,5} = 14.4$, $J_{5,6} = J_{5,6'} = 7.5$ Hz, H-5 of sphingosine), 5.68 (m, 1 H, H-8^{V}), 5.46 (m, 1 H, H-4 of sphingosine), 5.41 (d, 1 H, H-4^{IV}), 5.36 (dd, 1 H, $J_{1,2} = 7.8$, $J_{2,3} = 10.1$ Hz, H-2^{IV}), 5.10 (dd, 1 H, $J_{6,7} = 2.5$, $J_{7,8} = 9.4$ Hz, H-7^{V}), 4.98 (d, 1 H, $J_{1,2} = 7.8$ Hz, H-1^{IV}), 4.95 (dd, 1 H, $J_{1,2} = 8.0$, $J_{2,3} = 9.6$ Hz, H-2^{I}), 4.87 (dd, 1 H, $J_{1,2} = 8.0$, $J_{2,3} = 9.6$ Hz, H-2^{II}), 4.83 (dd, 1 H, $J_{2,3} = 10.0$, $J_{3,4} = 3.2$ Hz, H-3^{IV}), 4.61 (d, 1 H, $J_{1,2} = 8.0$ Hz, H-1^{III}), 4.42 (d, 1 H, $J_{1,2} = 7.8$ Hz, H-1^{II}), 4.28 (d, 1 H, $J_{1,2} = 8.0$ Hz, H-1^{I}), 4.02 (dd, 1 H, $J_{8,9'} = 6.4$, $J_{gem} = 11.4$ Hz, H-$9'^{V}$), 3.83 (s, 3 H, COO*Me*), 3.23 (br-d, 1 H, H-6^{III}), 2.50 (dd, 1 H, $J_{3eq,4} = 4.3$, $J_{gem} = 12.5$ Hz, H-$3^{V}eq$), 2.16, 2.12, 2.10, 2.07, 2.008, 2.001, 1.99, 1.96, 1.92, 1.89, 1.87 (11 s, 33 H, 11 AcO), 1.59 (t, 1 H, $J_{gem} = J_{3ax,4} = 12.5$ Hz, H-$3^{V}ax$), 1.47 (s, 3 H, AcN^{III}), 1.24 (s, 52 H, 26 CH_2), 0.87 (t, 6 H, $J_{vic} = 6.6$ Hz, 2 *Me*CH_2). ^{13}C NMR ($CDCl_3$): δ 172.66 (C=O), 171.86 (C=O), 170.67 (C=O), 170.50 (C=O), 170.12 (C=O), 170.02 (C=O), 169.66 (C=O), 169.27 (C=O), 168.62 (C=O), 168.42 (C=O), 167.88 (C=O), 166.41 (C=O), 165.76 (C=O), 165.54 (C=O), 165.51 (C=O), 165.19 (C=O), 164.92 (C=O), 137.59, 133.31, 133.40, 130.30, 129.99, 129.77, 129.61, 128.56, 128.42, 124.65 (arom-C), 101.28, 100.77, 100.37, 99.97, 96.85, 75.60, 75.21, 74.24, 74.09, 72.80, 72.35, 72.11, 71.72, 71.39, 71.04, 70.55, 69.04, 68.45, 67.99, 67.46, 67.02, 66.73, 62.84, 61.95, 61.50, 60.45, 54.89, 53.35, 50.65, 49.66, 37.28, 36.87, 32.35, 31.93, 29.71, 29.54, 29.50, 29.44, 29.37, 29.26, 28.97, 25.74, 23.26, 22.70, 21.38, 20.88, 20.74, 20.38, 20.13, 14.12. Anal. Calcd for $C_{124}H_{168}F_3N_3O_{46}$ (2492.09): C, 59.72; H, 6.79; N, 1.69. Found: C, 59.70; H, 6.79; N, 1.40.

4.18. Methyl 4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-trifluoroacetamido-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2-acetamido-3-*O*-acetyl-2-deoxy-6-*O*-sulfo-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-3-*O*-benzoyl-2-octadecanamido-4-octadecene-1,3-diol triethylammonium salt (21)

To a solution of **20** (16.2 mg, 6.5 μmol) in DMF (1.5 mL) was added sulfur trioxide·pyridine complex (6.3 mg, 39 μmol) and the mixture was stirred for 6 h at rt. Et_3N (0.1 mL) was added, and the mixture was concentrated. Column chromatography (1:1 $CHCl_3$–MeOH) of the residue on Sephadex LH-20 gave the crude sulfated product, and this was purified by column chromatography (30:1 $CHCl_3$–MeOH) on silica gel to afford **21** (12.2 mg, 70.2%) as an amorphous mass; $[\alpha]_D$ +2.9° (*c* 0.24, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.30–7.42 (m, 20 H, 4 Ph), 6.09 (d, 1 H, $J_{NH,2} = 10.1$ Hz, NH^{III}), 5.86 (dt, 1 H, $J_{4,5} = 14.4$, $J_{5,6} = J_{5,6'} = 7.5$ Hz, H-5 of sphingosine), 5.73 (d, 1 H, $J_{NH,2} = 9.4$ Hz, NH of sphingosine), 5.66 (m, 1 H, H-8^{V}), 5.47 (d, 1 H, H-4^{IV}), 5.39 (dd, 1 H, $J_{1,2} = 8.0$, $J_{2,3} = 10.1$ Hz, H-2^{IV}), 5.24 (dd, 1 H, $J_{6,7} = 2.5$, $J_{7,8} = 9.4$ Hz, H-7^{V}), 5.18 (d, 1

H, $J_{1,2}=8.0$ Hz, H-1^{IV}), 5.12 (t, 1 H, $J_{3,4}=9.4$ Hz, H-3^{I}), 4.98–4.93 (m, 2 H, H-3^{IV} and H-2^{II}), 4.86 (dd, 1 H, $J_{1,2}=8.0$, $J_{2,3}=9.4$ Hz, H-2^{I}), 4.42 (d, 1 H, $J_{1,2}=8.0$ Hz, H-1^{I}), 4.30 (d, 1 H, $J_{1,2}=7.8$ Hz, H-1^{III}), 4.25 (d, 1 H, $J_{1,2}=7.1$ Hz, H-1^{II}), 3.75 (s, 3 H, COO*Me*), 3.68 (t, 1 H, H-2^{III}), 3.13 (q, 6 H, 3NCH_2CH$_3$), 2.41 (dd, 1 H, $J_{3eq,4}=4.3$, $J_{gem}=12.3$ Hz, H-$3^{V}eq$), 2.09, 2.07, 2.04, 2.02, 2.00, 1.98, 1.96, 1.94, 1.89, 1.88, 1.76 (11 s, 33 H, 11 AcO), 1.68 (s, 3 H, AcN^{III}), 1.53 (t, 1 H, $J_{gem}=J_{3ax,4}=$ 12.3 Hz, H-$3^{V}ax$), 1.34 (t, 9 H, 3NCH$_2$*CH$_3$*), 1.25 (s, 52 H, 26 CH$_2$), 0.87 (t, 6 H, $J_{vic}=6.9$ Hz, 2 *Me*CH$_2$). Anal. Calcd for $C_{130}H_{183}F_3N_4O_{49}S$ (2673.16): C, 58.37; H, 6.90; N, 2.09. Found: C, 58.10; H, 6.75; N, 1.91.

4.19. 5-Amino-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid-(2→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-6-*O*-sulfo-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-2-octadecanamido-4-octadecene-1,3-diol disodium salt (22, GSC-516)

To a solution of **21** (12.2 mg, 4.56 μmol) in MeOH (4 mL) and dioxane (0.4 mL) was added a catalytic amount of 28% NaOMe in MeOH, and the mixture was stirred for 72 h at 45 °C. Water (0.1 mL) was added and the mixture was stirred for 24 h at 45 °C, and then concentrated. Column chromatography (1:2:1 $CHCl_3$–MeOH–H_2O) of the residue on Sephadex LH-20 gave the target molecule **22** (7.4 mg, quant) as an amorphous mass; $[\alpha]_D$ −18.4° (*c* 0.1, 1:2:1 $CHCl_3$–MeOH–H_2O); ^{1}H NMR (CD_3OD): δ 5.59 (dt, 1 H, $J_{4,5}=15.5$, $J_{5,6}=J_{5,6'}=8.0$ Hz, H-5 of sphingosine), 5.35 (dd, 1 H, $J_{3,4}=7.8$, $J_{4,5}=15.3$ Hz, H-4 of sphingosine), 4.57 (d, 1 H, $J_{1,2}=8.2$ Hz, H-1^{III}), 4.40 (dd, 1 H, $J_{1,2}=8.0$ Hz, H-1^{IV}), 4.27 (d, 1 H, $J_{1,2}=7.2$ Hz, H-1^{II}), 4.21 (d, 1 H, $J_{1,2}=7.8$ Hz, H-1^{I}), 4.12 (dd, 1 H, $J_{2,3}=10.5$, $J_{3,4}=4.3$ Hz, H-3^{IV}), 3.69 (dd, 1 H, $J_{1,2}=8.2$, $J_{2,3}=10.1$ Hz, H-2^{III}), 3.65 (dd, 1 H, H-3^{II}), 3.43 (dd, 1 H, H-2^{IV}), 2.83 (t, 1 H, H-5^{V}), 2.74 (dd, 1H, $J_{3eq,4}=4.3$ Hz, $J_{gem}=12.1$ Hz, H-$3^{V}eq$), 2.09 (t, 1 H, H-1′of stearoyl), 1.94 (dd, 1 H, H-6 of sphingosine), 1.89 (s, 3 H, AcN^{III}), 1.62 (t, 1 H, $J_{3ax,4}=J_{gem}=12.1$ Hz, H-$3^{V}ax$), 1.48 (m, 2 H, H-2′ of stearoyl), 1.29 (m, 1 H, H-6′ of sphingosine), 1.20 (s, 52 H, 26 CH$_2$), 0.81 (t, 6 H, $J_{vic}=6.9$ Hz, 2 *Me*CH$_2$). FABMS (negative-ion): Calcd for $C_{71}H_{127}N_3Na_2O_{33}S$: *m/z* 1627.7868; found: *m/z* 1581.9762 [M − 2Na]$^-$, 888 [lactosyl ceramide]$^-$, 726 [glucosyl ceramide]$^-$ and 564 [ceramide]$^-$.

4.20. 5-Amino-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl 1,5-lactam-(2→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-6-*O*-sulfo-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-2-octadecanamido-4-octadecene-1,3-diol sodium salt (23, GSC-550)

To a solution of **22** (3.2 mg, 1.96 μmol) in DMF (0.5 mL) was added HBTU (4.6 mg, 12 μmol) and HOBt (1 mg, 7.4 μmol), and the mixture was stirred for 2 h at 65 °C, and then concentrated. Column chromatography (1:2:1 $CHCl_3$–MeOH–H_2O) of the residue on Sephadex LH-20 gave **23** (3 mg, 96%) as an amorphous mass; $[\alpha]_D$ +18.3° (*c* 0.06, 1:2:1 $CHCl_3$–MeOH–H_2O); ^{1}H NMR (CD_3OD): δ 5.59 (dt, 1 H, $J_{4,5}=15.3$, $J_{5,6}=J_{5,6'}=8.5$ Hz, H-5 of sphingosine), 5.35 (dd, 1 H, $J_{3,4}=7.8$, $J_{4,5}=$ 15.3 Hz, H-4 of sphingosine), 4.58 (d, 1 H, $J_{1,2}=8.5$ Hz, H-1^{III}), 4.41 (d, 1 H, $J_{1,2}=7.8$ Hz, H-1^{IV}), 4.35 (br-d, 1 H, H-6^{V}), 4.27 (d, 1 H, $J_{1,2}=7.3$ Hz, H-1^{II}), 4.20 (d, 1 H, $J_{1,2}=7.8$ Hz, H-1^{I}), 4.10 (m, 2 H, H-1 of sphingosine), 4.09 (dd, 1 H, $J_{3,4}=4.1$ Hz H-3^{IV}), 3.46 (dd, 1 H, $J_{1,2}=7.8$, $J_{2,3}=10.3$ Hz, H-2^{IV}), 3.41 (t, 1 H, $J_{2,3}=9.4$ Hz, H-3^{I}), 3.32 (m, 1 H, H-8^{V}), 2.29 (dd, 1H, $J_{3\beta,4}=$ 10.5, $J_{gem}=14.1$ Hz, H-$3^{V}\beta$), 2.08 (t, 1 H, H-1′ of stearoyl), 2.01 (dd, 1H, $J_{3\alpha,4}=5.0$, $J_{gem}=14.1$ Hz, H-$3^{V}\alpha$), 1.92 (dd 1 H, H-6 of sphingosine), 1.88 (s, 3 H, AcN^{III}), 1.47 (m, 2 H, H-2′ of stearoyl), 1.29 (dd, 1 H, H-6′ of sphingosine), 1.19 (s, 52 H, 26 CH$_2$), 0.85 (t, 6 H, $J_{vic}=6.4$ Hz, 2 *Me*CH$_2$). FABMS (negative-ion): Calcd for $C_{71}H_{126}N_3NaO_{32}S$: *m/z* 1587.7943; found: *m/z* 1565.8483 [M − Na]$^-$, 888 [lactosyl ceramide]$^-$, 726 [glucosyl ceramide]$^-$ and 564 [ceramide]$^-$.

4.21. 2-(Trimethylsilyl)ethyl methyl 4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-trifluoroacetamido-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-6-*O*-4-methoxyphenyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (24)

To a solution of **13** (599 mg, 0.248 mmol) in dry CH_2Cl_2 (8 mL) were added Me_3SiCl (94 μL, 0.73 mmol), $SnCl_2$ (31 mg, 0.16 mmol), and anisole (40 μL, 0.36 mmol) at 0 °C, and the mixture was stirred for 1.5 h at 0 °C. After the completion of the reaction, the mixture was extracted with $CHCl_3$. The extract was successively washed with M Na_2CO_3 and water, dried (Na_2SO_4) and concentrated. Column chromatography (70:1 $CHCl_3$–MeOH) of the residue on silica gel afforded **24** (552 mg, 97%) as an amorphous mass; $[\alpha]_D$ +30.6° (*c* 0.18, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.13–6.51 (m, 53 H, 2 MeO*Ph*, 9 Ph), 6.12 (d, 1 H, $J_{5,NH}=9.4$ Hz, NH^{V}), 5.58 (m, 1 H, H-8^{V}), 5.55 (dd, 1 H, $J_{1,2}=8.0$, $J_{2,3}=9.8$ Hz, H-2^{IV}), 5.36 (d, 1H, H-4^{IV}), 5.19 (dd, 1 H, $J_{6,7}=2.1$,

$J_{7,8}=9.2$ Hz, H-7^{V}), 5.12 (d, 1 H, $J_{1,2}=8.0$ Hz, H-1^{IV}), 4.95 (dd, 1 H, H-3^{IV}), 4.91 (dd, 1 H, $J_{1,2}=7.8$, $J_{2,3}=$ 10.5 Hz, H-2^{I}), 4.39 (d, 1 H, $J_{1,2}=7.8$ Hz, H-1^{III}), 4.33 (d, 1 H, $J_{1,2}=7.8$ Hz, H-1^{I}), 3.89 (s, 3 H, COO*Me*), 3.75 (dd, 1 H, $J_{5,6}=10.8$, $J_{6,7}=2.1$ Hz, H-6^{V}), 3.68 (S, 3 H, *Me*OPh), 3.65 (dd, 1 H, H-2^{III}), 3.54 (m, 2 H, $Me_3SiCH_2CH_2$), 2.51 (dd, 1 H, $J_{3eq,4}=4.3$, $J_{gem}=12.3$ Hz, H-3^{V}*eq*), 2.20, 2.05, 1.90, 1.52 (4 s, 12 H, 4 AcO), 1.59 (t, 1 H, $J_{gem}=J_{3eq,4}=12.3$ Hz, H-3^{V}*ax*), 1.44 (s, 3 H, AcN), 1.01 (m, 2 H, $Me_3SiCH_2CH_2$). ^{13}C NMR ($CDCl_3$): δ 172.31 (C=O), 172.98 (2C=O), 171.69 (C=O), 171.15 (C=O), 169.27 (C=O), 167.59 (C=O), 167.04 (C=O), 166.62 (C=O), 155.22, 154.09, (MeO*Ph*), 140.59, 140.52, 140.45, 140.17, 139.74, 139.72, 134.99, 134.86, 134.76, 131.53, 131.42, 131.31, 130.80, 130.62, 130.37, 130.02, 129.98, 129.94, 129.71, 129.65, 129.62, 129.58, 129.39, 129.33, 129.12, 128.95, 128.92, 128.89, 128.76, 128.50, 128.47, 128.38 (arom-C), 117.03, 115.87, (MeO*Ph*), 104.47, 103.97, 103.73, 102.96, 98.37, 84.24, 83.76, 83.21, 81.16, 78.05, 77.48, 76.77, 76.41, 76.34, 76.03, 75.62, 74.99, 74.88, 74.59, 74.55, 74.36, 73.14, 72.85, 72.80, 72.30, 70.10, 69.78, 69.59, 68.86, 68.71, 67.81, 64.22, 63.54, 57.20, 57.00, 54.86, 50.86, 38.73, 24.33, 22.85, 22.24, 21.79, 21.63, 19.83. Anal. Calcd for $C_{121}H_{135}F_3N_2O_{37}Si$ (2292.85): C, 63.34; H, 5.93; N, 1.22. Found: C, 63.30; H, 5.87; N, 1.09.

4.22. 2-(Trimethylsilyl)ethyl methyl 4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-trifluoroacetamido-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-2-acetamido-2-deoxy-6-*O*-4-methoxyphenyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (26)

To a solution of **24** (520 mg, 0.22 mmol) and **25** (238 mg, 0.45 mmol) in dry benzene (13 mL) was added 4 Å MS (800 mg), and the mixture was stirred for 3 h at rt, then cooled to 0 °C. *N*-Iodosuccinimide (NIS; 435 mg, 1.92 mmol) and CF_3SO_3H (TfOH; 28 μL, 0.31 mmol) were added to the mixture, and it was stirred for 2 h at 7 °C, and then neutralized with Et_3N. After dilution with $CHCl_3$, the precipitate was filtered off, and washed with $CHCl_3$. The filtrate and washings was combined, and successively washed with M Na_2CO_3 and $Na_2S_2O_3$, dried (Na_2SO_4) and concentrated. Column chromatography (100:1 $CHCl_3$–MeOH) of the residue on silica gel afforded **26** (536.5 mg, 87%) as an amorphous mass; $[\alpha]_D$ −13.2° (*c* 0.64, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.19–6.69 (m, 64 H, MeO*Ph*, 12 Ph), 5.89 (1 H, $J_{5,NH}=$ 9.38 Hz, NH^{V}), 5.63 (m, 1 H, H-8^{V}), 5.46 (dd, 1 H, $J_{1,2}=8.0$, $J_{2,3}=10.0$ Hz, H-2^{IV}), 5.39 (d, 1 H, H-4^{IV}), 5.24 (d, 1H, H-4^{II}), 5.19 (dd, 1 H, H-7^{V}), 5.01 (1 H, $J_{1,2}=8.0$ Hz, H-1^{IV}), 5.00 (d, 1 H, $J_{1,2}=3.2$ Hz, H-1^{VI}), 4.98 (dd, 1 H, $J_{2,3}=10.0$, $J_{3,4}=3.4$ Hz, H-3^{IV}), 4.47 (d, 1 H, $J_{1,2}=8.0$ Hz, H-1^{III}), 4.43 (d, 1 H, $J_{1,2}=7.8$ Hz, H-1^{II}), 4.36 (d, 1 H, $J_{1,2}=8.0$ Hz, H-1^{I}), 3.86 (s, 3 H, COO*Me*), 3.69 (s, 3 H, *Me*OPh), 3.41 (dd, 1 H, $J_{1,2}=$ 8.0, $J_{2,3}=9.2$ Hz, H-2^{III}), 3.36 (m, 2 H, $Me_3SiCH_2CH_2$), 2.54 (dd, 1 H, $J_{3eq,4}=4.6$, $J_{gem}=12.5$ Hz, H-3^{V}*eq*), 2.17, 1.93, 1.91, 1.51 (4 S, 12 H, 4 AcO), 1.70 (t, 1 H, $J_{gem}=J_{3eq,4}=12.5$ Hz, H-3^{V}*ax*), 1.49 (s, 3 H, AcN), 1.04 (m, 2 H, $Me_3SiCH_2CH_2$), 0.88 (d, 3 H, $J_{5,6}=6.4$ Hz, H-6^{VI}). ^{13}C NMR ($CDCl_3$): δ 171.68 (C=O), 170.89 (C=O), 170.68 (C=O), 170.68 (C=O), 168.93 (C=O), 167.76 (C=O), 167.47 (C=O), 167.08 (C=O), 166.86 (C=O), 155.16, 153.72, (MeO*Ph*), 140.56, 140.37, 140.19, 139.83, 139.76, 139.64, 139.55, 139.45, 134.93, 134.64, 134.24, 131.30, 131.06, 130.80, 130.60, 130.40, 130.07, 129.74, 129.61, 129.39, 129.35, 129.32, 129.27, 129.21, 129.16, 129.10, 129.06, 128.95, 128.68, 128.63, 128.48, 128.27, 128.12, 127.75, (arom-C), 116.42, 115.78, (MeO*Ph*), 104.12, 103.34, 100.23, 97.94, 97.77, 95.47, 85.57, 84.24, 83.24, 80.01, 79.83, 79.26, 76.92, 76.29, 75.99, 75.79, 75.01, 74.31, 74.23, 73.98, 73.44, 72.31, 69.91, 69.66, 69.51, 68.40, 68.00, 67.49, 63.35, 56.72, 54.55, 50.84, 38.37, 23.76, 22.48, 21.62, 21.50, 21.28, 19.60, 17.94. Anal. Calcd for $C_{148}H_{163}F_3N_2O_{41}Si$ (2709.05): C, 65.57; H, 6.06; N, 1.03. Found: C, 65.40; H, 5.91; N, 0.74.

4.23. 2-(Trimethylsilyl)ethyl methyl 4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-trifluoroacetamido-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)]-2-acetamido-2-deoxy-6-*O*-4-methoxyphenyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (27)

A solution of **26** (300 mg, 0.11 mmol) in EtOH (40 mL) and HOAc (8 mL) was vigorously stirred with $Pd(OH)_2$ (300 mg) for 48 h at rt under hydrogen. The catalyst was collected and washed with MeOH. The combined filtrate and washings was concentrated, and the residue was treated with Ac_2O (6 mL) and pyridine (10 mL) for 12 h at rt, then cooled to 0 °C. MeOH (5 mL) was added, the mixture was concentrated, and the residue was extracted with $CHCl_3$ and successively washed with cold 2 M HCl and water, dried (Na_2SO_4) and concentrated. Column chromatography (50:1 $CHCl_3$–MeOH) of the residue on silica gel gave **27** (252 mg, quant) as an amorphous mass; $[\alpha]_D$ −5.2° (*c* 0.4, $CHCl_3$) ^{1}H NMR ($CDCl_3$): δ 8.27–6.80 (m, 19 H, MeOPh, 3 Ph), 6.08 (d, 1 H, $J_{NH,2}=9.2$ Hz, NH^{III}), 5.79 (d, 1 H, $J_{NH,5}=9.8$ Hz, NH^{V}), 5.74 (m, 1 H, H-8^{V}), 5.50 (dd, 1 H, $J_{1,2}=8.2$, $J_{2,3}=10.0$ Hz, H-2^{IV}), 5.38 (d, 1 H, H-4^{II}), 5.25 (d, 1 H, H-4^{IV}), 5.18 (dd, 1 H, $J_{6,7}=2.1$, $J_{7,8}=9.2$ Hz, H-7^{V}), 5.05 (dd, 1 H, $J_{2,3}=10.1$, $J_{3,4}=3.4$ Hz, H-3^{IV}), 5.01 (d, 1 H, $J_{1,2}=8.2$ Hz, H-1^{IV}), 4.96 (dd, 1 H, H-3^{II}), 4.93 (d, 1 H, $J_{1,2}=2.9$ Hz, H-1^{VI}), 4.88 (dd, 1 H, $J_{1,2}=8.0$,

$J_{2,3}=10.3$ Hz, H-2^{II}), 4.84 (dd, 1 H, $J_{1,2}=8.0$, $J_{2,3}=$ 10.0 Hz, H-2^{I}), 4.48 (d, 1H, $J_{1,2}=8.0$ Hz, H-1^{II}), 4.42 (dd, 1 H, $J_{8,9'}=5.7$, $J_{gem}=11.7$ Hz, H-9′^{II}), 4.16 (d, 1 H, $J_{1,2}=8.0$ Hz, H-1^{I}), 3.88 (s, 3 H, COO*Me*), 3.81 (dd, 1 H, $J_{1,2}=7.6$, $J_{2,3}=10.7$ Hz, H-2^{III}), 3.73 (s, 3 H, MeOPh), 3.50 (m, 2 H, $Me_3SiCH_2CH_2$), 2.48 (dd, 1 H, $J_{3eq,4}=4.8$, $J_{gem}=12.5$ Hz, H-3^{V}*eq*), 2.21 2.14, 2.07, 2.06, 2.04, 2.03, 2.02, 2.00, 1.99, 1.95, 1.93, 1.91, 1.90 (13 s, 39 H, 13 AcO), 1.66 (t, 1H, $J_{gem}=J_{3ax,4}=12.5$ Hz, H-3^{V}*ax*), 1.60 (s, 3 H, AcN^{III}), 0.88 (m, 2 H, $Me_3SiCH_2CH_2$), 0.80 (d, 3 H, $J_{5,6}=6.2$ Hz, H-6^{VI}). ^{13}C NMR ($CDCl_3$): δ 172.47 (C=O), 172.31 (C=O), 172.26 (C=O), 172.05 (C=O), 171.97 (C=O), 171.77 (C=O), 171.70 (C=O), 171.50 (2C=O), 171.38 (C=O), 171.15 (C=O), 171.09 (C=O), 171.03 (C=O), 170.16 (C=O), 169.27 (C=O), 167.83 (C=O), 167.51 (C=O), 167.32 (C=O), 155.48, 154.17, (MeO*Ph*), 135.05, 134.86, 134.67, 131.97, 131.30, 131.06, 131.00, 130.73, 130.52, 130.44, 130.02, 129.75, 129.64 (arom-C), 116.81, 116.19 (MeO*Ph*), 102.78, 102.15, 101.32, 100.12, 98.40, 96.92, 79.05, 77.87, 77.46, 76.53, 74.19, 73.96, 73.18, 72.79, 72.54, 72.42, 72.36, 71.54, 70.25, 70.00, 69.09, 68.91, 68.81, 68.72, 68.51, 68.22, 66.44, 64.10, 63.95, 63.81, 57.06, 55.34, 54.89, 50.89, 38.57, 24.38, 22.87, 22.26, 22.18, 22.42, 22.08, 22.02, 21.97, 21.89, 21.82, 21.74, 19.29, 16.96. Anal. Calcd for $C_{103}H_{127}F_3N_2O_{50}Si$ (2276.72): C, 54.30; H, 5.62; N, 1.23. Found: C, 54.09; H, 5.53; N, 1.06.

4.24. 2-(Trimethylsilyl)ethyl methyl 4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-trifluoroacetamido-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (28)

To a solution of **27** (252 mg, 0.11 mmol) in MeCN (8.1 mL) and water (0.9 mL) was added CAN (190 mg, 0.33 mmol), and the mixture was stirred for 1.5 h at 0 °C and extracted with AcOEt. The extract was successively washed with M Na_2CO_3 and water, dried (Na_2SO_4) and concentrated. Column chromatography (40:1 $CHCl_3$–MeOH) of the residue on silica gel gave **28** (204 mg, 85%) as an amorphous mass; $[\alpha]_D$ −20.1° (*c* 0.38, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.23–7.42 (m, 15 H, 3 Ph), 6.06 (d, 1 H, $J_{NH,2}=9.2$ Hz, NH^{III}), 5.71 (m, 1 H, H-8^{V}), 5.41 (dd, 1 H, $J_{1,2}=8.5$, $J_{2,3}=10.7$ Hz, H-2^{IV}), 5.40 (d, 1 H, H-4^{II}), 5.33 (d, 1 H, H-4^{IV}), 5.21 (dd, 1 H, $J_{2,3}=10.6$, $J_{3,4}=3.2$ Hz, H-3^{IV}), 5.12 (d, 1 H, $J_{1,2}=2.7$ Hz, H-1^{VI}), 4.96 (dd, 1 H, $J_{1,2}=2.7$, $J_{2,3}=7.1$ Hz, H-2^{VI}), 4.88 (dd, 1 H, $J_{1,2}=8.0$, $J_{2,3}=9.6$ Hz, H-2^{II}), 4.46 (d, 1 H, $J_{1,2}=8.0$ Hz, H-1^{II}), 4.30 (d, 1 H, $J_{1,2}=7.8$ Hz, H-1^{I}), 4.04 (dd, 1 H, $J_{8,9'}=6.2$, $J_{gem}=11.3$ Hz, H-9′^{V}), 3.96 (dd, 1 H, $J_{8,9}=7.0$, $J_{gem}=11.3$ Hz, H-9^{V}), 3.83 (s, 3 H, COO*Me*), 3.57 (m, 2 H, $Me_3SiCH_2CH_2$), 3.22 (br-d, H-6^{III}), 2.45 (dd, 1 H, $J_{3eq,4}=4.3$, $J_{gem}=12.5$ Hz, H-3^{V}*eq*), 2.15, 2.107, 2.103, 2.099, 2.097, 2.04, 2.03, 2.02, 2.01, 2.00, 1.92, 1.90, 1.82 (13 s, 39 H, 13 AcO), 1.64 (t, 1 H, $J_{gem}=J_{3ax,4}=12.5$ Hz, H-3^{V}*ax*), 1.54 (s, 3 H, AcN^{III}), 1.20 (d, 3H, $J_{5,6}=6.4$ Hz, H-6^{VI}), 0.93 (m, 2 H, $Me_3SiCH_2CH_2$). ^{13}C NMR ($CDCl_3$): δ 172.75 (C=O), 172.72 (C=O), 172.41 (C=O), 172.12 (C=O), 172.07 (C=O), 172.01 (C=O), 171.94 (C=O), 171.57 (C=O), 171.43 (C=O), 171.33 (C=O), 171.06 (C=O), 170.84 (C=O), 170.77 (C=O), 170.55 (C=O), 169.22 (C=O), 167.83 (C=O), 167.26 (C=O), 166.62 (C=O), 134.81, 134.61, 134.51, 131.69, 131.50, 131.28, 131.12, 131.05, 130.15, 130.01, 129.79 (arom-C), 102.13, 101.94, 101.70, 101.38, 98.44, 96.65, 77.35, 77.19, 75.42, 74.20, 74.02, 73.11, 73.02, 72.65, 72.35, 72.30, 72.19, 70.51, 69.99, 69.86, 69.44, 69.00, 68.93, 68.31, 66.01, 64.07, 63.61, 63.02, 62.95, 62.31, 54.77, 50.94, 38.68, 31.12, 24.79, 22.81, 22.39, 22.26, 22.18, 22.09, 22.01, 21.84, 21.68, 19.29, 17.32. Anal. Calcd for $C_{96}H_{121}F_3N_2O_{49}Si$ (2170.68): C, 53.08; H, 5.61; N, 1.29. Found: C, 52.82; H, 5.43; N, 1.17.

4.25. 2-(Trimethylsilyl)ethyl methyl 4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-trifluoroacetamido-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)]-2-acetamido-2-deoxy-6-*O*-levulinoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (29)

To a solution of **28** (204 mg, 93.9 μmol) in pyridine (6 mL) was added levulinic anhydride (50 mg, 0.23 mmol) and DMAP (20 mg, 0.16 mmol), and the mixture was stirred for 48 h at 65 °C, then cooled to 0 °C. MeOH (3 mL) was added, the mixture was concentrated, and the residue was extracted with $CHCl_3$ and successively washed with cold 2 M HCl and water, dried (Na_2SO_4) and concentrated. Column chromatography (50:1 $CHCl_3$–MeOH) of the residue on silica gel gave **29** (174.8 mg, 82%) as an amorphous mass; $[\alpha]_D$ −13.8° (*c* 0.44, $CHCl_3$),^{1}H NMR ($CDCl_3$): δ 8.16–7.46 (m, 15 H, 3 Ph), 6.33 (d, $J_{NH,2}=8.9$ Hz, NH^{III}), 5.64 (m, 1 H, H-8^{V}), 5.46 (d, 1 H, $J_{NH,5}=9.8$ Hz, NH^{V}), 5.42 (dd, 1 H, $J_{1,2}=8.2$, $J_{2,3}=10.1$ Hz, H-2^{IV}), 5.36 (m, 1 H, H-5^{V}), 5.24 (dd, 1 H, $J_{6,7}=2.1$, $J_{7,8}=9.8$ Hz, H-7^{V}), 5.21 (d, 1 H, H-4^{IV}), 5.13 (t, 1 H, $J_{2,3}=9.6$ Hz, H-3^{I}), 5.07 (d, 1 H, $J_{1,2}=2.9$ Hz, H-1^{VI}), 4.96 (dd, 1 H, $J_{2,3}=10.1$, $J_{3,4}=$ 3.9 Hz, H-3^{IV}), 4.93 (d, 1 H, $J_{1,2}=8.2$ Hz, H-1^{IV}), 4.90 (dd, 1 H, $J_{1,2}=7.8$, $J_{2,3}=9.8$ Hz, H-2^{II}), 4.85 (dd, 1 H, $J_{1,2}=8.0$, $J_{2,3}=9.6$ Hz, H-2^{I}), 4.77 (m, 1 H, H-5^{VI}), 4.45 (d, 1H, $J_{1,2}=8.0$ Hz, H-1^{I}), 4.42 (d, 1H, $J_{1,2}=7.8$ Hz, H-1^{II}), 4.33 (d, 1H, $J_{1,2}=7.8$ Hz, H-1^{II}), 4.29 (dd, 1 H, $J_{8,9'}=4.3$ Hz, H-9′^{V}), 4.05 (dd, 1 H, $J_{8,9}=4.9$, $J_{gem}=$ 11.4 Hz H-9^{V}), 3.80 (s, 3 H, COO*Me*), 3.71 (dd, 1 H,

$J_{2,3}=9.4$ Hz, H-2^{III}), 3.56 (m, 2 H, $Me_3SiCH_2CH_2$), 2.70–2.53 (m, 4 H, MeCOCH_2CH_2) 2.44 (dd, 1 H, $J_{3eq,4}=4.6$, $J_{gem}=12.5$ Hz, H-3^{V}eq), 2.13, 2.11, 2.089, 2.087, 2.081, 2.07, 2.04, 2.03, 2.01, 1.988, 1.985, 1.92, 1.88, 1.81 (14 s, 42 H, 13 AcO and MeCOCH$_2$CH$_2$), 1.63 (t, 1H, $J_{gem}=J_{3ax,4}=12.5$ Hz, H-3^{V}ax), 1.55 (s, 3 H, AcN^{III}), 1.20 (d, 3 H, $J_{5,6}=6.6$ Hz, H-6^{VI}), 0.92 (m, 2 H, Me_3SiCH_2CH$_2$). ^{13}C NMR ($CDCl_3$): δ 208.37 (C=O), 173.45 (C=O), 172.63 (C=O), 172.33 (C=O), 172.20 (C=O), 172.03 (2C=O), 171.95 (C=O), 171.92 (C=O), 171.49 (C=O), 171.36 (C=O), 171.16 (C=O), 171.07 (C=O), 171.03 (C=O), 170.82 (C=O), 170.66 (C=O), 169.21 (C=O), 167.93 (C=O), 167.34 (C=O), 166.47 (C=O), 134.83, 134.61, 134.57, 131.65, 131.63, 131.26, 131.14, 131.06, 130.99, 130.15, 129.99, 129.86 (arom-H), 102.05, 101.98, 101.39, 101.05, 98.43, 96.54, 77.31, 76.10, 74.58, 74.19, 74.07, 73.24, 73.13, 73.06, 72.61, 72.57, 72.50, 72.19, 70.69, 70.04, 70.00, 69.76, 69.42, 68.90, 68.67, 67.69, 66.04, 63.73, 63.53, 63.35, 63.12, 62.95, 58.83, 54.80, 51.04, 39.13, 38.55, 31.21, 31.12, 29.24, 24.79, 22.81, 22.34, 22.26, 22.19, 22.14, 22.11, 22.08, 22.01, 21.84, 21.70, 19.30, 17.34. Anal. Calcd for $C_{101}H_{127}F_3N_2O_{51}Si$ (2268.71): C, 53.44; H, 5.64; N, 1.23. Found: C, 53.18; H, 5.38; N, 1.04.

4.26. Methyl 4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-trifluoroacetamido-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)]-2-acetamido-2-deoxy-6-*O*-levulinoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate (30)

The 2-(trimethylsilyl)ethyl group of **29** (174.8 mg, 77 μmol) was removed by treatment with CF_3CO_2H (3 mL) in CH_2Cl_2 (4 mL) for 3 h at rt. AcOEt (2 mL) was added, and the mixture was concentrated. Column chromatography (50:1 $CHCl_3$–MeOH) of the residue on silica gel gave the 1-OH free derivative (153.5 mg, 94%). This compound was treated with trichloroacetonitrile (214 μL, 17.1 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU; 11 μL, 0.068 mmol) in CH_2Cl_2 (5 mL) for 2 h at 0 °C. The mixture was concentrated, and the residue was chromatographed (40:1 $CHCl_3$–MeOH) on a column of silica gel to give the trichloroacetimidate **30** (154.9 mg, 87% in two steps) as an amorphous mass; $[\alpha]_D$ −4.2° (*c* 0.72, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.64 (s, 1 H, NH of imidate), 8.61–7.45 (m, 15 H, 3 Ph), 6.47 (d, 1 H, $J_{1,2}=3.7$ Hz, H-1^{I}), 6.24 (d, 1 H, $J_{NH,2}=8.9$ Hz NH^{III}), 5.64 (m, 1 H, H-8^{V}), 5.50 (t, 1 H, $J_{2,3}=9.6$ Hz, H-3^{I}), 5.42 (dd, 1 H, $J_{1,2}=8.5$, $J_{2,3}=10.3$ Hz, H-2^{IV}), 5.24 (dd, 1 H, $J_{6,7}=2.1$ Hz, H-7^{V}), 5.22 (d, 1 H, H-4^{IV}), 5.07 (d, 1 H, $J_{1,2}=2.9$ Hz, H-1^{VI}), 5.04 (dd, 1 H, $J_{1,2}=3.7$, $J_{2,3}=10.1$ Hz, H-2^{I}), 4.97 (dd, 1 H, $J_{3,4}=4.1$ Hz, H-3^{IV}), 4.92 (dd, 1 H, $J_{1,2}=7.8$, $J_{2,3}$ 9.8 Hz, H-2^{II}), 4.90 (d, 1 H, $J_{1,2}=8.5$ Hz, H-1^{IV}), 4.79 (m, 1 H, H-5^{VI}), 4.37 (d, 1H, $J_{1,2}=7.8$ Hz, H-1^{II}), 4.29 (dd, 1 H, $J_{8,9'}=3.9$ Hz, H-9$'^{V}$), 3.80 (s, 3 H, COOMe), 2.70–2.52 (m, 4 H, MeCOCH_2CH_2) 2.44 (dd, 1 H, $J_{3eq,4}=4.6$, $J_{gem}=12.5$ Hz, H-3^{V}eq), 2.13, 2.11, 2.09, 2.08, 2.079, 2.076, 2.075, 2.03, 2.01, 1.99, 1.98, 1.93, 1.88, 1.80 (14 s, 42 H, 13 AcO and MeCOCH$_2$CH$_2$), 1.63 (t, 1H, $J_{gem}=J_{3ax,4}=12.5$ Hz, H-3^{V}ax), 1.55 (s, 3 H, AcN^{III}), 1.21 (d, 3 H, $J_{5,6}$ 6.4 Hz, H-6^{VI}). Anal. Calcd for $C_{98}H_{115}Cl_3F_3N_3O_{51}$ (2311.55): C, 50.86; H, 5.01; N, 1.82. Found: C, 50.82; H, 4.75; N, 1.65.

4.27. Methyl 4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-trifluoroacetamido-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)]-2-acetamido-2-deoxy-6-*O*-levulinoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol (31)

To a solution of **30** (154.9 mg, 67 μmol) and **6** (60 mg, 139 μmol) in dry CH_2Cl_2 (1 mL) were added 4 Å MS (type AW300; 2.5 g), and the mixture was stirred for 2 h at rt and then cooled to 0 °C. TMSOTf (1.33 μL, 6.8 μmol) was added to the mixture, and this was stirred for 48 h at 0 °C, neutralized with Et_3N and filtered. Chromatography (60:1 $CHCl_3$–MeOH) of the residue on silica gel afforded **31** (119.3 mg, 69%) as an amorphous mass; $[\alpha]_D$ −17.2° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.17–7.44 (m, 20 H, 4 Ph), 6.02 (d, 1 H, $J_{NH,2}=8.7$ Hz, NH^{III}), 5.91 (dt, 1 H, $J_{4,5}=14.8$, $J_{5,6}=J_{5,6'}=6.6$ Hz, H-5 of sphingosine), 5.67 (m, 1 H, H-8^{V}), 5.60 (dd, 1 H, $J_{3,4}=4.1$, $J_{4,5}=8.2$ Hz, H-4^{VI}), 5.52 (m, 1 H, H-4 of sphingosine), 5.42 (dd, 1 H, $J_{1,2}=7.8$, $J_{2,3}=9.8$ Hz, H-2^{IV}), 5.36 (d, 1 H, H-4^{II}), 5.25 (dd, 1 H, $J_{7,8}=7.3$ Hz, H-7^{V}), 5.23–5.21 (m, 2 H, H-3^{II} and H-4^{IV}), 5.14 (t, 1 H, $J_{2,3}=9.2$ Hz, H-3^{I}), 5.08 (d, 1 H, $J_{1,2}=2.9$ Hz, H-1^{VI}), 4.97 (dd, 1 H, $J_{2,3}=10.9$, $J_{3,4}=4.1$ Hz, H-3^{IV}), 4.93–4.88 (m, 5 H, H-4^{V}, $J_{1,2}=8.9$ H-1^{III}, $J_{1,2}=7.8$ H-1^{IV}, H-2 ^{IV} and H-2^{I}), 4.79 (m, 1 H, H-5^{VI}), 4.50 (d, 1 H, $J_{1,2}=7.8$ Hz, H-1^{I}), 4.33 (d, 1 H, $J_{1,2}=7.9$ Hz, H-1^{II}), 4.04 (dd, 1 H, $J_{8,9'}=5.9$, $J_{gem}=11.4$ Hz, H-9$'^{V}$), 3.82 (s, 3 H, COOMe), 3.71 (dd, 1 H, H-2^{III}), 2.71–2.54 (m, 4 H, MeCOCH_2CH_2), 2.44 (dd, 1 H, $J_{3eq,4}=4.3$, $J_{gem}=12.5$ Hz, H-3^{V}eq), 2.14, 2.12, 2.097, 2.091, 2.07, 2.06, 2.04, 2.02, 2.01, 2.00, 1.99, 1.93, 1.90, 1.82 (14 s, 42 H, 13 AcO and Me-COCH$_2$CH$_2$), 1.65 (t, 1 H, $J_{gem}=J_{3ax,4}=12.3$ Hz, H-3^{V}ax), 1.55 (s, 3 H, AcN^{III}), 1.37 (m, 1 H, H-6 of sphingosine), 1.23 (s, 22 H, 11 CH$_2$), 1.21 (d, 3 H, $J_{5,6}=6.4$ Hz, H-6^{VI}), 0.88 (t, 3 H, $J_{vic}=6.9$ Hz, MeCH$_2$). ^{13}C NMR ($CDCl_3$): δ 206.93 (C=O), 172.03 (C=O), 171.19 (C=O), 170.90 (C=O), 170.77 (C=O), 170.62 (C=O),

170.55 (C=O), 170.47 (C=O), 170.36 (C=O), 170.06 (C=O), 169.89 (C=O), 169.74 (C=O), 169.61 (C=O), 169.58 (C=O), 169.39 (C=O), 169.30 (C=O), 167.76 (C=O), 166.49 (C=O), 165.88 (C=O), 165.08 (C=O), 165.02 (C=O), 139.06, 133.39, 133.23, 130.22, 130.09, 129.89, 129.83, 129.74, 129.63, 129.54, 128.72, 128.57, 128.46, 128.42, 122.57, 100.74, 100.56, 100.40, 99.57, 96.97, 95.11, 75.88, 75.66, 74.66, 73.12, 72.83, 72.58, 71.80, 71.64, 71.36, 71.22, 71.03, 70.73, 69.25, 68.52, 68.37, 67.98, 67.02, 66.20, 64.60, 63.50, 62.09, 61.87, 61.65, 61.50, 53.41, 49.69, 37.70, 37.11, 32.39, 31.92, 29.79, 29.65, 29.59, 29.40, 29.35, 29.16, 28.72, 27.80, 23.37, 22.69, 21.40, 20.92, 20.82, 20.74, 20.70, 20.59, 20.39, 20.27, 15.91, 14.13. Anal. Calcd for $C_{121}H_{152}F_3N_5O_{53}$ (2579.93): C, 56.30; H, 5.93; N, 2.71. Found: C, 56.12; H, 5.90; N, 2.46.

4.28. Methyl 4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-trifluoroacetamido-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)]-2-acetamido-2-deoxy-6-*O*-levulinoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-3-*O*-benzoyl-2-octadecanamido-4-octadecene-1,3-diol (32)

H_2S was bubbled through a stirred solution of **31** (119.3 mg, 46 μmol) in pyridine (5 mL) and water (1 mL) for 72 h at 0 °C. The mixture was concentrated, and the residual syrup was treated with octadecanoic acid (40 mg, 0.14 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (WSC; 26 mg, 0.13 mmol) in CH_2Cl_2 (2 mL) 24 h at rt. The mixture was extracted with $CHCl_3$, and the extract was successively washed with M HCl and water, dried (Na_2SO_4) and concentrated. Column chromatography (50:1 $CHCl_3$–MeOH) of the residue on silica gel gave **32** (59.9 mg, 46%) as an amorphous mass; $[\alpha]_D$ −18.0° (*c* 0.14, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.25–7.41 (m, 20 H, 4 Ph), 5.97 (d, 1 H, $J_{NH,2}$ = 9.2 Hz, NH^{III}), 5.86 (dt, 1 H, $J_{4,5}$ = 14.8, $J_{5,6}$ = $J_{5,6'}$ = 6.6 Hz, H-5 of sphingosine), 5.73 (d, 1 H, $J_{NH,2}$ = 9.2 Hz, NH of sphingosine), 5.66 (m, 1 H, H-8^V), 5.53 (m, 1 H, H-3 of sphingosine), 5.46 (m, 1 H, H-4 of sphingosine), 5.42 (dd, 1 H, $J_{1,2}$ = 8.5, $J_{2,3}$ = 10.3 Hz, H-2^{IV}), 5.25 (dd, 1 H, $J_{6,7}$ = 2.3 Hz, H-7^V), 5.21 (d, 1 H, H-4^{IV}), 5.13 (t, 1 H, $J_{2,3}$ = 9.4 Hz, H-3^I), 5.08 (d, 1 H, $J_{1,2}$ = 2.9 Hz, H-1^{VI}), 4.96 (dd, 1 H, $J_{2,3}$ = 10.9, $J_{3,4}$ 4.1 Hz, H-3^{IV}), 4.90 (d, 1 H, $J_{1,2}$ = 7.8 Hz, H-1^{IV}), 4.86 (dd, 1 H, $J_{1,2}$ = 8.0, $J_{2,3}$ = 9.8 Hz, H-2^I), 4.49 (m, 1 H, H-2 of sphingosine), 4.43 (d, 1 H, $J_{1,2}$ = 7.8 Hz, H-1^{II}), 4.30 (d, 1 H, $J_{1,2}$ = 8.0 Hz, H-1^I), 3.96 (dd, 1 H, H-1′ of sphingosine), 3.82 (s, 3 H, COO*Me*), 3.75 (dd, 1 H, H-2^{III}), 3.60 (dd, 1 H, $J_{1,2}$ = 4.6, J_{gem} = 10.1 Hz, H-1 of sphingosine), 2.71–2.55 (m, 4 H, MeCO*CH*$_2$*CH*$_2$), 2.45 (dd, 1 H, $J_{3eq,4}$ = 4.6, J_{gem} = 12.3 Hz, H-3^V*eq*), 2.14, 2.12, 2.094, 2.092, 2.090, 2.04, 2.01, 1.998, 1.993, 1.991, 1.93, 1.91, 1.90, 1.82 (14 s, 42 H, 13 AcO and *Me*COCH$_2$CH$_2$), 1.64 (t, 1 H, J_{gem} = $J_{3ax,4}$ = 12.3 Hz, H-3^V*ax*), 1.56 (s, 3 H, AcN^{III}), 1.23 (s, 52 H, 26 CH$_2$), 1.21 (d, 3 H, $J_{5,6}$ 6.6 Hz, H-6^{VI}), 0.88 (t, 6 H, J_{vic} = 6.6 Hz, 2 *Me*CH$_2$). ^{13}C NMR ($CDCl_3$): δ 206.86 (C=O), 172.69 (C=O), 172.05 (C=O), 171.20 (C=O), 170.90 (C=O), 170.76 (C=O), 170.60 (C=O), 170.51 (C=O), 170.45 (C=O), 170.33 (C=O), 170.07 (C=O), 169.83 (C=O), 169.76 (C=O), 169.69 (C=O), 169.63 (C=O), 169.39 (C=O), 169.20 (C=O), 167.79 (C=O), 166.52 (C=O), 165.91 (C=O), 165.20 (C=O), 165.04 (C=O), 137.64, 133.15, 133.07, 130.26, 129.91, 129.87, 129.63, 129.37, 129.14, 128.75, 128.59, 128.43, 128.37, 128.28, 127.71, 126.40, 124.66, 101.39, 100.57, 100.37, 99.76, 96.99, 95.12, 75.93, 75.55, 74.08, 73.47, 73.44, 73.16, 72.88, 72.33, 71.84, 71.69, 71.28, 71.12, 70.77, 70.08, 69.40, 69.19, 68.53, 68.02, 67.68, 67.46, 67.04, 66.25, 64.64, 62.14, 61.99, 61.92, 53.44, 50.65, 49.76, 37.74, 37.16, 36.89, 32.38, 31.96, 30.07, 29.74, 29.57, 29.52, 29.47, 29.39, 29.29, 28.98, 27.85, 27.11, 25.77, 23.39, 22.72, 21.41, 20.94, 20.78, 20.70, 20.60, 20.41, 20.30, 15.94, 14.15. Anal. Calcd for $C_{139}H_{188}F_3N_3O_{54}$ (2820.20): C, 59.16; H, 6.71; N, 1.49. Found: C, 59.05; H, 6.43; N, 1.31.

4.29. 5-Acetylamino-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl 1,5-lactam-(2→3)-β-D-galactopyranosyl-(1→4)-[α-L-fucopyranosyl-(1→3)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-2-octadecanamido-4-octadecene-1,3-diol (37, GSC-517)

To a solution of **32** (34.9 mg, 12 μmol) in MeOH (5 mL) was added a catalytic amount of 28% NaOMe in MeOH, and the mixture was stirred for 72 h at 45 °C. Water (0.2 mL) was added, and the mixture was stirred for 24 h at 45 °C and then concentrated. Column chromatography (1:2:1 $CHCl_3$–MeOH–H_2O) of the residue on Sephadex LH-20 gave the *N*-deacetyl saialyl Le^x ganglioside **35** (21 mg, quant) as an amorphous mass. To a solution of **35** (3.1 mg, 1.8 μmol) in DMF (1 mL) was added HBTU (4.4 mg, 11 μmol) and HOBt (1.2 mg, 8 μmol), and the mixture was stirred for 2 h at 65 °C, and then concentrated. Column chromatography (1:3:1 $CHCl_3$–MeOH–H_2O) of the residue on Sephadex LH-20 gave lactamized sialyl Le^x ganglioside **37** (2.9 mg, 94.7%) as an amorphous mass; $[\alpha]_D$ +1.7° (*c* 0.05, 1:2:1 $CHCl_3$–MeOH–H_2O); ^{1}H NMR (CD_3OD): δ 5.58 (dt, 1 H, $J_{4,5}$ = 15.1, $J_{5,6}$ = $J_{5,6'}$ = 7.3 Hz, H-5 of sphingosine), 5.37 (dd, 1 H, $J_{3,4}$ = 7.8, $J_{4,5}$ = 15.1 Hz, H-4 of sphingosine), 4.96 (d, 1 H, $J_{1,2}$ = 3.9 Hz, H-1^{VI}), 4.59 (d, 1 H, $J_{1,2}$ = 8.7 Hz, H-1^{III}), 4.41 (d, 1 H, $J_{1,2}$ = 8.0 Hz, H-1^{IV}), 4.32 (br-d, 1 H, H-6^V), 4.26 (d, 1 H, $J_{1,2}$ = 7.3 Hz, H-1^{II}), 4.21 (d, 1 H, $J_{1,2}$ = 8.0 Hz, H-1^I), 4.08 (dd, 1 H, $J_{2,3}$ = 9.8, $J_{3,4}$ = 4.1 Hz, H-3^{II}), 4.02 (m, 1 H, H-4^V), 3.96

(dd, 1 H, H-3^{IV}), 3.83 (dd, 1 H, $J_{2,3}=9.4$ Hz, H-2^{III}), 3.70 (dd, 1 H, $J_{6,7}=2.3$, $J_{7,8}=10.6$ Hz, H-7^{V}), 3.53 (dd, 1 H, $J_{2,3}=9.4$ Hz, H-2^{IV}), 3.49 (dd, 1 H, $J_{2,3}=10.8$ Hz, H-2^{II}), 3.43 (t 1 H, $J_{2,3}=9.2$ Hz, H-3^{I}), 3.18 (dd, 1 H, $J_{2,3}=9.4$ Hz, H-2^{I}), 2.29 (dd, 1H, $J_{3\beta,4}=10.8$, $J_{gem}=$ 14.1 Hz, H-3$^{V}\beta$), 2.07 (t, 1 H, H-1′ of stearoyl), 2.01 (dd, 1H, $J_{3\alpha,4}=4.1$, $J_{gem}=14.1$ Hz, H-3$^{V}\alpha$), 1.92 (m, 1 H, H-6′ of sphingosine), 1.88 (s, 3 H, AcN^{III}), 1.48 (m, 1 H, H-2′ of stearoyl), 1.28 (dd, 1 H, H-6 of sphingosine), 1.19 (s, 52 H, 26 CH_2), 1.08 (d, 3 H, $J_{5,6}=6.9$ Hz, H-6^{VI}), 0.80 (t, 6 H, $J_{vic}=6.4$ Hz, 2 *Me*CH_2); FABMS (negative-ion): Calcd for $C_{77}H_{137}N_3O_{33}$: *m*/z 1631.9134; found: *m*/z 1630.9056 $[M-H]^-$, 1399 $[M-Neu]^-$, 1237 [1399-Gal]$^-$, 888 [lactosyl ceramide]$^-$, 726 [glucosyl ceramide]$^-$and 564 [ceramide]$^-$.

4.30. Methyl 4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-trifluoroacetamido-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-3-*O*-benzoyl-2-octadecanamido-4-octadecene-1,3-diol (33)

To a solution of **32** (59.9 mg, 21.2 μmol) in EtOH (3 mL) was added hydrazine acetate (10 mg, 0.108 mmol), and the mixture was stirred for 4 h at rt and then concentrated. Column chromatography (60:1 $CHCl_3$–MeOH) of the residue on silica gel gave **33** (40.47 mg, 70%) as an amorphous mass; $[\alpha]_D$ −12.8° (*c* 0.80, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.21–7.43 (m, 20 H, 4 Ph), 6.02 (d, 1 H, $J_{NH,2}=8.7$ Hz, NH^{III}), 5.87 (dt, 1 H, $J_{4,5}=15.1$, $J_{5,6}=J_{5,6'}=7.1$ Hz, H-5 of sphingosine), 5.74 (d, 1 H, $J_{NH,2}=9.2$ Hz, NH of sphingosine), 5.71 (m, 1 H, H-8^{V}), 5.54 (m, 1 H, H-3 of sphingosine), 5.45 (m, 1 H, H-4 of sphingosine), 5.41 (dd, 1 H, $J_{2,3}=10.1$ Hz, H-2^{IV}), 5.40 (d, 1 H, H-4^{II}), 5.34 (d, 1 H, H-4^{IV}), 5.22 (1 H, $J_{6,7}=2.9$, $J_{7,8}=10.9$ Hz, H-7^{V}), 5.13 (dd, 1 H, $J_{2,3}=9.8$ Hz, H-3^{VI}), 5.12 (d, 1 H, $J_{1,2}=3.4$ Hz, H-1^{VI}), 4.95 (dd 1 H, H-2^{VI}), 4.88 (dd, 1 H, $J_{2,3}=9.4$ Hz, H-2^{I}), 4.43 (d, 1 H, $J_{1,2}=7.8$ Hz, H-1^{II}), 4.27 (d, 1 H, $J_{1,2}=7.8$ Hz, H-1^{I}), 3.84 (s, 3 H, COO*Me*), 3.21 (br-d, 1 H, H-6^{III}), 2.47 (dd, 1 H, $J_{3eq,4}=4.6$, $J_{gem}=12.5$ Hz, H-3^{V}*eq*), 2.16, 2.15, 2.12, 2.109, 2.103, 2.09, 2.08, 2.05, 2.02, 2.01, 2.008, 2.002, 1.99 (13 s, 39 H, 13 AcO), 1.65 (t, 1 H, $J_{gem}=J_{3ax,4}=12.5$ Hz, H-3^{V}*ax*), 1.56 (s, 3 H, AcN^{III}), 1.26 (s, 52 H, 26 CH_2), 0.88 (t, 9 H, $J_{vic}=6.4$ Hz, 2 *Me*CH_2 and H-6^{VI}). ^{13}C NMR ($CDCl_3$): δ 172.66 (C=O), 172.03 (C=O), 171.18 (C=O), 170.87 (C=O), 170.74 (C=O), 170.58 (C=O), 170.43 (C=O), 170.30 (C=O), 170.05 (2C=O), 169.74 (2C=O), 169.67 (C=O), 169.36 (C=O), 169.17 (C=O), 167.76 (C=O), 166.50 (C=O), 165.88 (C=O), 165.18 (C=O), 165.02 (C=O), 137.62, 133.13, 133.04, 130.23, 129.89, 129.84, 129.61, 129.34, 129.11, 128.73, 128.57, 128.40, 128.34, 128.26, 127.69, 126.46, 124.63 (arom-C),101.39, 100.57, 100.37, 99.76, 96.99, 95.12, 77.99, 75.90, 75.52, 74.06, 73.44, 73.14, 72.85, 72.30, 72.20, 71.82, 71.67, 71.25, 71.09, 70.75, 70.06, 69.38, 68.50, 67.99, 67.02, 66.23, 64.61, 62.12, 61.90, 53.41, 50.62, 49.74, 37.71, 37.14, 36.86, 32.35, 31.93, 30.36, 30.04, 29.72, 29.54, 29.50, 29.44, 29.37, 29.26, 28.96, 27.82, 27.08, 25.74, 23.37, 22.69, 21.39, 20.92, 20.75, 20.67, 20.58, 20.39, 20.27, 15.92, 14.13. Anal. Calcd for $C_{134}H_{182}F_3N_3O_{52}$ (2722.16): C, 59.09; H, 6.73; N, 1.54. Found: C, 58.79; H, 6.49; N, 1.49.

4.31. Methyl 4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-trifluoroacetamido-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)]-2-acetamido-2-deoxy-6-*O*-sulfo-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-3-*O*-benzoyl-2-octadecanamido-4-octadecene-1,3-diol triethylammonium salt (34)

To a solution of **33** (40.8 mg, 14.8 μmol) in DMF (2 mL) was added sulfur trioxide–pyridine complex (14.4 mg, 90 μmol), and the mixture was stirred for 6 h at rt. Et_3N (0.1 mL) was added and the mixture was concentrated. Column chromatography (1:1 $CHCl_3$–MeOH) of the residue on Sephadex LH-20 gave the crude sulfated product, and this was purified by column chromatography (10:1 $CHCl_3$–MeOH) on silica gel to afford **34** (34.9 mg, 81%) as an amorphous mass; $[\alpha]_D$ −19.5° (*c* 0.12, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.24–7.42 (m, 20 H, 4 Ph), 6.48 (br-d, NH^{III}), 5.86 (dt, 1 H, $J_{4,5}=14.1$, $J_{5,6}=J_{5,6'}=7.6$ Hz, H-5 of sphingosine), 5.74 (d, 1 H, $J_{NH,2}=$ 8.9 Hz, NH of sphingosine), 5.70 (m, 1 H, H-8^{V}), 5.53 (m, 1 H, H-3 of sphingosine), 5.45 (m, 1 H, H-4 of sphingosine), 5.27 (dd, 1 H, $J_{7,8}=10.1$ Hz, H-7^{V}), 5.23–5.18 (m, 2 H, H-2^{IV} and H-1^{VI}), 5.14–5.10 (m, 2 H, H-4^{IV} and H-3^{II}), 4.92 (dd, 1 H, $J_{1,2}=7.6$, $J_{2,3}=9.4$ Hz, H-2^{I}), 4.87 (dd, 1 H, $J_{1,2}=7.8$, $J_{2,3}=9.8$ Hz, H-2^{II}), 4.81 (dd, 1 H, $J_{2,3}=9.6$ Hz, H-3^{IV}), 4.60 (dd, 1 H, $J_{gem}=$ 12.4 Hz, H-6'^{III}), 4.47 (m, 1 H, H-2 of sphingosine), 4.42 (d, 1 H, $J_{1,2}=7.8$ Hz, H-1^{II}), 4.38 (m, 1 H, H-6^{III}), 4.28 (d, 1 H, $J_{1,2}=7.6$ Hz, H-1^{I}), 4.12 (m, 1 H, H-5^{III}), 3.99 (dd, 1 H, $J_{1',2}$ 3.9, $J_{gem}=10.0$ Hz, H-1′ of sphingosine), 3.95–3.93 (m, 2 H, H-4^{III} and H-9^{V}), 3.84 (s, 3 H, COO*Me*), 3.62 (dd, 1 H, $J_{1,2}=4.3$, $J_{gem}=10.0$ Hz, H-1 of sphingosine), 3.15 (q, 6 H, 3 NCH_2CH$_3$), 2.56 (dd, 1 H, $J_{3eq,4}=4.1$, $J_{gem}=12.1$ Hz, H-3^{V}*eq*), 2.20, 2.16, 2.15, 2.14, 2.10, 2.08, 2.07, 2.01, 1.99, 1.96, 1.94, 1.92, 1.91 (13 s, 39 H, 13 AcO), 1.59 (t, 1 H, $J_{gem}=J_{3ax,4}=12.1$ Hz, H-3^{V}*ax*), 1.49 (s, 3 H, AcN^{III}), 1.35 (t, 9 H, 3 NCH$_2$*CH$_3$*), 1.24 (s, 52 H, 26 CH_2), 0.88 (t, 9 H,

$J_{vic} = 6.6$ Hz, 2 *Me*CH_2 and H-6^{VI}). Anal. Calcd for $C_{140}H_{197}F_3N_4O_{55}S$ (2903.24): C, 57.88; H, 6.83; N, 1.93. Found: C, 57.82; H, 6.57; N, 1.72.

4.32. 5-Amino-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid-(2→3)-β-D-galactopyranosyl-(1→4)-[α-L-fucopyranosyl-(1→3)]-2-acetamido-2,6-deoxy-6-*O*-sulfo-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-2-octadecanamido-4-octadecene-1,3-diol disodium salt (36, GSC-406)

To a solution of **34** (34.9 mg, 12 μmol) in MeOH (5 mL) and dioxane (0.4 mL) was added a catalytic amount of 28% NaOMe in MeOH, and the mixture was stirred for 72 h at 45 °C. Water (0.2 mL) was added, and the mixture was stirred for 24 h at 45 °C and then concentrated. Column chromatography (1:2:1 $CHCl_3$–MeOH–H_2O) of the residue on Sephadex LH-20 gave **36** (21 mg, quant) as an amorphous mass; $[\alpha]_D$ −25.4° (*c* 0.2, 1:2:1 $CHCl_3$–MeOH–H_2O); ^{1}H NMR (CD_3OD): δ 5.68 (dt, 1 H, $J_{4,5} = 15.5$, $J_{5,6} = J_{5,6'} = 8.9$ Hz, H-5 of sphingosine), 5.42 (dd, 1 H, $J_{3,4} = 7.5$, $J_{4,5} = 15.5$ Hz, H-4 of sphingosine), 5.05 (d, 1 H, $J_{1,2} = 3.7$ Hz, H-1^{VI}), 4.56 (d, 1 H, $J_{1,2} = 7.8$ Hz, H-1^{III}), 4.35 (d, 1 H, $J_{1,2} =$ 8.0 Hz, H-1^{IV}), 4.28 (d, 1 H, $J_{1,2} = 7.8$ Hz, H-1^{II}), 4.21 (m, 1 H, H-1′ sphingosine), 4.08 (d, 1 H, $J_{1,2} = 8.0$ Hz, H-1^{I}), 3.96 (m, 1 H, H-6^{V}), 3.89 (dd, 1 H, $J_{3,4} = 3.9$ Hz, H-3^{IV}), 3.69 (m, 1 H, H-4^{V}), 3.64 (dd, 1 H, $J_{1,2} = 8.0$ Hz, H-2^{IV}), 3.53 (dd, 1 H, H-2^{III}), 3.41 (m, 1 H, H-8^{V}), 3.31 (dd, 1 H, H-2^{II}), 3.09 (t, 1 H, H-5^{V}), 2.81 (dd, 1H, $J_{3eq,4} = 4.3$, $J_{gem} = 12.1$ Hz, H-3^{V}*eq*), 2.16 (t, 1 H, H-1′ of stearoyl), 2.01 (dd, 1 H, H-6 of sphingosine), 1.96 (s, 3 H, AcN^{III}), 1.79 (t, 1 H, $J_{3ax,4} = J_{gem} = 12.1$ Hz, H-3^{V}*ax*), 1.57 (m, 2 H, H-2′ of stearoyl), 1.35 (dd, 1 H, H-6′ of sphingosine), 1.30 (s, 52 H, 26 CH_2), 1.16 (d, 3 H, $J_{5,6} = 6.4$ Hz, H-6^{VI}), 0.89 (t, 6 H, $J_{vic} = 6.6$ Hz, 2 *Me*CH_2). Anal. Calcd for $C_{77}H_{137}N_3Na_2O_{37}S$ (1773.84): C, 52.10; H, 7.78; N, 2.37. Found: C, 52.01; H, 7.69; N, 2.18.

4.33. 5-Amino-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl 1,5-lactam-(2→3)-β-D-galactopyranosyl-(1→4)-[α-L-fucopyranosyl-(1→3)]-2-acetamido-2-deoxy-6-*O*-sulfo-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-2-octadecanamido-4-octadecene-1,3-diol sodium salt (38, GSC-535)

To a solution of **36** (8 mg, 4.5 μmol) in DMF (1 mL) was added HBTU (10.56 mg, 27 μmol) and HOBt (2.5 mg, 18 μmol), and the mixture was stirred for 2 h at 65 °C and then concentrated. Column chromatography (1:2:1 $CHCl_3$–MeOH–H_2O) of the residue on Sephadex LH-20 gave the lactamized 6-sulfo sLe^x ganglioside **38** (7.4 mg, 94%) as an amorphous mass; $[\alpha]_D$ −7.35° (*c* 0.14, 1:2:1 $CHCl_3$–MeOH–H_2O); ^{1}H NMR (CD_3OD): δ 5.59 (dt, 1 H, $J_{4,5} = 15.2$, $J_{5,6} = J_{5,6'} = 8.2$ Hz, H-5 of sphingosine), 5.36 (dd, 1 H, $J_{3,4} = 7$ .8, $J_{4,5} = 15.2$ Hz, H-4 of sphingosine), 4.96 (d, 1 H, $J_{1,2} = 4.1$ Hz, H-1^{VI}), 4.66 (d, 1 H, $J_{1,2} = 7.8$ Hz, H-1^{III}), 4.49 (d, 1 H, $J_{1,2} =$ 7.8 Hz, H-1^{IV}), 4.37 (br-d, 1 H, H-6^{V}), 4.26 (d, 1 H, $J_{1,2} = 7.6$ Hz, H-1^{II}), 4.20 (d, 1 H, $J_{1,2} = 7.8$ Hz, H-1^{I}), 4.10 (m, 2 H, H-1′ of sphingosine), 4.01 (dd, 1 H, H-3^{IV}), 3.98–3.97 (m, 2 H, H-4^{V} and H-3 of sphingosine), 3.88 (m, 1 H, H-2 of sphingosine), 3.85 (dd, 1 H, H-2^{III}), 3.81 (dd, 1 H, H-3^{VI}), 3.75 (dd, 1 H, $J_{6,7} = 3.7$ Hz, H-7^{V}), 3.60 (m, 1 H, H-4^{VI}), 3.57 (dd, 1 H, $J_{1,2} = 7.8$, $J_{2,3} = 9.4$ Hz, H-2^{II}), 3.55 (dd, 1 H, $J_{2,3} = 10.2$ Hz, H-2^{VI}), 3.49 (dd 1 H, $J_{1,2} = 7.8$, $J_{2,3} = 10.7$ Hz, H-2^{IV}), 3.47 (m, 1 H, H-1 of sphingosine), 3.45 (m, 1 H, H-5^{V}), 3.42 (t, 1 H, H-3^{I}), 3.32 (m, 1 H, H-8^{V}), 3.18 (dd, 1 H, H-2^{I}), 2.30 (dd, 1H, $J_{3\beta,4} = 10.3$, $J_{gem} = 13.7$ Hz, H-3$^{V}\beta$), 2.08 (t, 1 H, H-1′ of stearoyl), 2.00 (dd, 1H, $J_{3\alpha,4} = 4.6$, $J_{gem} =$ 13.7 Hz, H-3$^{V}\alpha$), 1.93 (dd 1 H, $J_{5,6} = 8.2$, $J_{gem} = 13.9$ Hz, H-6′ of sphingosine), 1.88 (s, 3 H, AcN^{III}), 1.48 (m, 1 H, H-2′ of stearoyl), 1.29 (dd, 1 H, H-6 of sphingosine), 1.19 (s, 52 H, 26 CH_2), 1.07 (d, 3 H, $J_{5,6} = 6.4$ Hz, H-6^{VI}), 0.81 (t, 6 H, $J_{vic} = 6.6$ Hz, 2 *Me*CH_2). FABMS (negative-ion): Calcd for $C_{77}H_{136}N_3NaO_{36}S$: *m*/z 1733.8522; Found: *m*/z 1710.8624 $[M - Na]^-$, 1479 $[M -$ Lactamized Neu$]^-$, 1317 [1479-Gal]$^-$, 1237 $[M - SO_3Na$-Lactamized Neu-Gal$]^-$ 888 [lactosyl ceramide]$^-$, 726 [glucosyl ceramide]$^-$ and 564 [ceramide]$^-$.

4.34. Methods for TLC-immunostaining

Reactivities of lactamized-sialyl 6-sulfo Lewis X ganglioside **1** and the derivative **2** with G159 mAb were examined by a TLC-immunostaining method reported by Kannagi.[24] Glycolipids (1 or 2 μg) were chromatographed on an HPTLC plate (SiHPF, J. T. Baker Chemical Co., Phillipsburg, NJ) with a solvent system of 55:40:10 $CHCl_3$–MeOH–0.5% $CaCl_2$. After drying at rt, the TLC plates were soaked in 5% BSA–PBS for 2 h to block nonspecific binding of antibodies. The plates were then gently washed in 0.5% BSA–PBS with two changes of buffer and incubated with G159 mAb in 0.5% BSA–PBS at 4 °C for overnight. After three washings with 0.5% BSA–PBS, the plates were reacted with HRP-labeled goat anti-mouse IgG (Zymed Laboratories, Inc., South San Francisco, CA) diluted 1:1000 in 0.5% BSA–PBS for 1 h at rt. Positive reaction was visualized using ECL Western blotting detection reagents (Amersham Biosciences Ltd., Buckinghamshire, UK). Glycolipid spots in the control plates were visualized by orcinol–H_2SO_4 reagent.

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